INTERNATIONAL SEARCH REPORT

rnational application No.
PCT/US 00/11956

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-23 all partially

Remark on Pr test

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-23 all partially

   Polynucleotides from corn encoding COI1 polypeptides,and COI1 polypeptides as specified in SEQ ID NOS:1,2,and 15-18,chimeric genes, methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection based on said sequences.

2. Claims: 1-23 all partially

   Polynucleotides from rice encoding COI1 polypeptides,and COI1 polypeptides as specified in SEQ ID NOS:3,4,19 and 20,chimeric genes,methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection based on said sequences.

3. Claims: 1-23 all partially

   Polynucleotides from soybean encoding COI1 polypeptides,and COI1 polypeptides as specified in SEQ ID NOS:5,6,21 and 22,chimeric genes, methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection based on said sequences.

4. Claims: 1-23 all partially

   Polynucleotides from wheat encoding COI1 polypeptides,and COI1 polypeptides as specified in SEQ ID NOS:7,8,and 23-28,chimeric genes, methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection based on said sequences.

5. Claims: 1-24 all partially

   Polynucleotides from rice encoding Lls1 polypeptides,and Lls1 polypeptides as specified in SEQ ID NOS: 9,10,29 and 30,chimeric genes, methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection, and method for evaluating the ability of a compound to inhibit Lls1, based on said sequences.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

6. Claims: 1-24 all partially

Polynucleotides from soybean encoding Lls1 polypeptides,and Lls1 polypeptides as specified in SEQ ID NOS:11,12,31 and 32,chimeric genes, methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection, and method for evaluating the ability of a compound to inhibit Lls1, based on said sequences.

7. Claims: 1-24 all partially

Polynucleotides from wheat encoding Lls1 polypeptides,and Lls1 polypeptides as specified in SEQ ID NOS:13, 14,33-36,chimeric genes, methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection, and method for evaluating the ability of a compound to inhibit Lls1, based on said sequences.

INTERNATIONAL SEARCH REPORT

[redacted]tional Application No
PCT/US 00/11956

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C12N15/29 C07K14/415 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EMBL, BIOSIS, EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL [Online] ACCESSION NO:AQ161346, 9 September 1998 (1998-09-09) WING R.A.: "nbxb0006F06f CUGI Rice BAC Library Oryza sativa genomic clone nbxb0006F06f, genomic survey sequence." XP002146944 see sequence ---	1-4,16, 18
P,X	DATABASE EMBL [Online] ACCESSION NO:AW061660, 6 October 1999 (1999-10-06) WALBOT V.: "660012G08.y1 660 - Mixed stages of anther and pollen Zea mays cDNA, mRNA sequence" XP002146880 see sequence --- -/--	1-4,16, 18

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 September 2000	22. 12. 2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	MADDOX, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	DATABASE EMBL [Online] ACCESSION NO:AW054624, 26 September 1999 (1999-09-26) WALBOT V.: "660012G08.x1 660 - Mixed stages of anther and pollen Zea mays cDNA, mRNA sequence." XP002146945 see sequence --- DATABASE EMBL [Online] ACCESSION NO:AF036340, 29 May 1998 (1998-05-29) FEYS B.J., ET AL.: "Arabidopsis thaliana LRR-containing F-box protein (COI1) mRNA, complete cds." XP002146881 see sequence -& XIE, D.-X., ET AL.: "COI1: An Arabidsopsis gene required for jasmonate-regulated defense and fertility" SCIENCE, vol. 280, 15 May 1998 (1998-05-15), pages 1091-1094, XP002146875 the whole document -& FEYS, B.J., ET AL.: "ARABIDOPSIS MUTANTS SELECTED FOR RESISTANCE TO THE PHYTOTOXIN CORONATINE ARE MALE STERILE, INSENSITIVE TO METHYL JASMONATE AND RESISTANT TO A BACTERIAL PATHOGEN" THE PLANT CELL, vol. 6, 1994, pages 751-759, XP002049621 the whole document	1-4,16, 18 1-23
A	DATABASE EMBL [Online] ACCESSION NO:AI444738, 16 March 1999 (1999-03-16) WALBOT, V.: "486015G10.x5 486 - leaf primordia cDNA library from Hake lab Zea mays cDNA, mRNA sequence" XP002146974 see sequence --- DATABASE EMBL [Online] ACCESSION NO:AU032235, 19 October 1998 (1998-10-19) SASKAI, T.: "Oryza sativa cDNA, partial sequence (R3783_1A)." XP002146975 see sequence --- -/--	1-10 1-10

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BENEDETTI CELSO E ET AL: "Differential expression of a novel gene in response to coronatine, methyl jasmonate, and wounding in the Coi1 mutant of arabidopsis." PLANT PHYSIOLOGY (ROCKVILLE), vol. 116, no. 3, March 1998 (1998-03), pages 1037-1042, XP002146876 ISSN: 0032-0889 the whole document	1-23
A	WO 98 00023 A (KAZAN KEMAL ;MANNERS JOHN MICHAEL (AU); BROEKAERT WILLEM FRANS (BE) 8 January 1998 (1998-01-08) claim 7	23
A	WO 91 18512 A (UNIV WASHINGTON) 12 December 1991 (1991-12-12) the whole document	23
A	PENNINCKX IRIS A M A ET AL: "Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in arabidopsis." PLANT CELL, vol. 10, no. 12, December 1998 (1998-12), pages 2103-2113, XP002146877 ISSN: 1040-4651 the whole document	23
A	THOMMA BART P H J ET AL: "Separate jasmonate-dependent and salicylate-dependent defense-response pathways in Arabidopsis are essential for resistance to distinct microbial pathogens." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 95, no. 25, December 1998 (1998-12), pages 15107-15111, XP002146878 Dec., 1998 ISSN: 0027-8424 the whole document	23
A	CORDERO MARIA JOSE ET AL: "Expression of a maize proteinase inhibitor gene is induced in response to wounding and fungal infection: Systemic wound-response of a monocot gene." PLANT JOURNAL, vol. 6, no. 2, 1994, pages 141-150, XP002146879 ISSN: 0960-7412	

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	GRAY J ET AL: "A NOVEL SUPPRESSOR OF CELL DEATH IN PLANTS ENCODED BY THE LLS 1 GENE OF MAIZE" CELL,US,CELL PRESS, CAMBRIDGE, NA, vol. 89, 4 April 1997 (1997-04-04), pages 25-31, XP002068010 ISSN: 0092-8674 -& DATABASE EMBL [Online] ACCESSION NO:U77346, 18 April 1997 (1997-04-18) GRAY, J. ET AL.: "Zea mays lethal leaf-spot 1 (lls1) gene, partial cds." XP002068011 -& DATABASE EMBL [Online] ACCESSION NO:U77345, 18 April 1997 (1997-04-18) GRAY, J. ET AL.: "Zea mays lethal leaf-spot 1 (lls1) mRNA, partial cds." XP002146987 abstract --- WO 98 39422 A (GRAY JOHN ;PIONEER HI BRED INT (US); UNIV MISSOURI (US); BRIGGS ST) 11 September 1998 (1998-09-11) -----	

5

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/11956

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9800023 A	08-01-1998	AU 3183597 A	21-01-1998
		BR 9710000 A	10-08-1999
		EP 0912096 A	06-05-1999
WO 9118512 A	12-12-1991	AT 142420 T	15-09-1996
		AU 650459 B	23-06-1994
		AU 7953191 A	31-12-1991
		CA 2083595 A	26-11-1991
		DE 69122100 D	17-10-1996
		DE 69122100 T	06-02-1997
		DK 532650 T	07-10-1996
		EP 0532650 A	24-03-1993
		ES 2091930 T	16-11-1996
		GR 3021974 T	31-03-1997
		US 5935809 A	10-08-1999
		US 5883076 A	16-03-1999
		US 5378819 A	03-01-1995
WO 9839422 A	11-09-1998	AU 6678098 A	22-09-1998
		BR 9808161 A	28-03-2000
		EP 0981605 A	01-03-2000

PATENT COOPERATION TREATY

RECEIVED
JAN 02 2001
PATENT RECORDS CENTER

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

JAN 03 2001

To:
E.I. DU PONT DE NEMOURS AND COMPANY
Legal/Patent Records Center
Attn. Geiger, Kathleen W.
1007 Market Street
Wilmington, Delaware 19898
UNITED STATES OF AMERICA

KL

Date of mailing (day/month/year): 22/12/2000

Applicant's or agent's file reference: BB1356 PCT

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No.: PCT/US 00/11956

International filing date (day/month/year): 03/05/2000

Applicant: E.I. DU PONT DE NEMOURS AND COMPANY

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority:
European Patent Office, P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer: Nathalie Ostwinkel

REY NOTED

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
BB1356 PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 00/ 11956	03/05/2000	07/05/1999
Applicant		
E.I. DU PONT DE NEMOURS AND COMPANY		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 8 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☒ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readble form.

☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/11956

Box I Obs rvations where c rtain claims wer found unsearchable (Continuati n f it m 1 of first sh et)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-23 all partially

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-23 all partially

   Polynucleotides from corn encoding COI1 polypeptides,and COI1 polypeptides as specified in SEQ ID NOS:1,2,and 15-18,chimeric genes, methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection based on said sequences.

2. Claims: 1-23 all partially

   Polynucleotides from rice encoding COI1 polypeptides,and COI1 polypeptides as specified in SEQ ID NOS:3,4,19 and 20,chimeric genes,methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection based on said sequences.

3. Claims: 1-23 all partially

   Polynucleotides from soybean encoding COI1 polypeptides,and COI1 polypeptides as specified in SEQ ID NOS:5,6,21 and 22,chimeric genes, methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection based on said sequences.

4. Claims: 1-23 all partially

   Polynucleotides from wheat encoding COI1 polypeptides,and COI1 polypeptides as specified in SEQ ID NOS:7,8,and 23-28,chimeric genes, methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection based on said sequences.

5. Claims: 1-24 all partially

   Polynucleotides from rice encoding Lls1 polypeptides,and Lls1 polypeptides as specified in SEQ ID NOS: 9,10,29 and 30,chimeric genes, methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection, and method for evaluating the ability of a compound to inhibit Lls1, based on said sequences.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

6. Claims: 1-24 all partially

Polynucleotides from soybean encoding Lls1 polypeptides,and Lls1 polypeptides as specified in SEQ ID NOS:11,12,31 and 32,chimeric genes, methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection, and method for evaluating the ability of a compound to inhibit Lls1, based on said sequences.

7. Claims: 1-24 all partially

Polynucleotides from wheat encoding Lls1 polypeptides,and Lls1 polypeptides as specified in SEQ ID NOS:13, 14,33-36,chimeric genes, methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection, and method for evaluating the ability of a compound to inhibit Lls1, based on said sequences.

INT NATIONAL SEARCH REPORT

International Application No
PCT/US 00/11956

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C12N15/29 C07K14/415 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EMBL, BIOSIS, EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	✓DATABASE EMBL [Online] ACCESSION NO:AQ161346, 9 September 1998 (1998-09-09) WING R.A.: "nbxb0006F06f CUGI Rice BAC Library Oryza sativa genomic clone nbxb0006F06f, genomic survey sequence." XP002146944 see sequence ---	1-4,16, 18
P,X	✓DATABASE EMBL [Online] ACCESSION NO:AW061660, 6 October 1999 (1999-10-06) WALBOT V.: "660012G08.y1 660 - Mixed stages of anther and pollen Zea mays cDNA, mRNA sequence" XP002146880 see sequence --- -/--	1-4,16, 18

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 September 2000	22. 12. 2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	MADDOX, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	DATABASE EMBL [Online] ACCESSION NO:AW054624, 26 September 1999 (1999-09-26) WALBOT V.: "660012G08.x1 660 - Mixed stages of anther and pollen Zea mays cDNA, mRNA sequence." XP002146945 see sequence ---	1-4,16, 18
A	DATABASE EMBL [Online] ACCESSION NO:AF036340, 29 May 1998 (1998-05-29) FEYS B.J., ET AL.: "Arabidopsis thaliana LRR-containing F-box protein (COI1) mRNA, complete cds." XP002146881 see sequence -& XIE, D.-X., ET AL.: "COI1: An Arabidopsis gene required for jasmonate-regulated defense and fertility" SCIENCE, vol. 280, 15 May 1998 (1998-05-15), pages 1091-1094, XP002146875 the whole document -& FEYS, B.J., ET AL.: "ARABIDOPSIS MUTANTS SELECTED FOR RESISTANCE TO THE PHYTOTOXIN CORONATINE ARE MALE STERILE, INSENSITIVE TO METHYL JASMONATE AND RESISTANT TO A BACTERIAL PATHOGEN" THE PLANT CELL, vol. 6, 1994, pages 751-759, XP002049621 the whole document ---	1-23
A	DATABASE EMBL [Online] ACCESSION NO:AI444738, 16 March 1999 (1999-03-16) WALBOT, V.: "486015G10.x5 486 - leaf primordia cDNA library from Hake lab Zea mays cDNA, mRNA sequence" XP002146974 see sequence ---	1-10
A	DATABASE EMBL [Online] ACCESSION NO:AU032235, 19 October 1998 (1998-10-19) SASKAI, T.: "Oryza sativa cDNA, partial sequence (R3783_1A)." XP002146975 see sequence --- -/--	1-10

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BENEDETTI CELSO E ET AL: "Differential expression of a novel gene in response to coronatine, methyl jasmonate, and wounding in the Coil mutant of arabidopsis." PLANT PHYSIOLOGY (ROCKVILLE), vol. 116, no. 3, March 1998 (1998-03), pages 1037-1042, XP002146876 ISSN: 0032-0889 the whole document	1-23
A	WO 98 00023 A (KAZAN KEMAL ;MANNERS JOHN MICHAEL (AU); BROEKAERT WILLEM FRANS (BE) 8 January 1998 (1998-01-08) claim 7	23
A	WO 91 18512 A (UNIV WASHINGTON) 12 December 1991 (1991-12-12) the whole document	23
A	PENNINCKX IRIS A M A ET AL: "Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in arabidopsis." PLANT CELL, vol. 10, no. 12, December 1998 (1998-12), pages 2103-2113, XP002146877 ISSN: 1040-4651 the whole document	23
A	THOMMA BART P H J ET AL: "Separate jasmonate-dependent and salicylate-dependent defense-response pathways in Arabidopsis are essential for resistance to distinct microbial pathogens." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 95, no. 25, December 1998 (1998-12), pages 15107-15111, XP002146878 Dec., 1998 ISSN: 0027-8424 the whole document	23
A	CORDERO MARIA JOSE ET AL: "Expression of a maize proteinase inhibitor gene is induced in response to wounding and fungal infection: Systemic wound-response of a monocot gene." PLANT JOURNAL, vol. 6, no. 2, 1994, pages 141-150, XP002146879 ISSN: 0960-7412	

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	GRAY J ET AL: "A NOVEL SUPPRESSOR OF CELL DEATH IN PLANTS ENCODED BY THE LLS 1 GENE OF MAIZE" CELL,US,CELL PRESS, CAMBRIDGE, NA, vol. 89, 4 April 1997 (1997-04-04), pages 25-31, XP002068010 ISSN: 0092-8674	
✓	-& DATABASE EMBL [Online] ACCESSION NO:U77346, 18 April 1997 (1997-04-18) GRAY, J. ET AL.: "Zea mays lethal leaf-spot 1 (lls1) gene, partial cds." XP002068011	
✓	-& DATABASE EMBL [Online] ACCESSION NO:U77345, 18 April 1997 (1997-04-18) GRAY, J. ET AL.: "Zea mays lethal leaf-spot 1 (lls1) mRNA, partial cds." XP002146987 abstract	

✓	WO 98 39422 A (GRAY JOHN ;PIONEER HI BRED INT (US); UNIV MISSOURI (US); BRIGGS ST) 11 September 1998 (1998-09-11)	

5

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9800023 A	08-01-1998	AU 3183597 A	21-01-1998
		BR 9710000 A	10-08-1999
		EP 0912096 A	06-05-1999
WO 9118512 A	12-12-1991	AT 142420 T	15-09-1996
		AU 650459 B	23-06-1994
		AU 7953191 A	31-12-1991
		CA 2083595 A	26-11-1991
		DE 69122100 D	17-10-1996
		DE 69122100 T	06-02-1997
		DK 532650 T	07-10-1996
		EP 0532650 A	24-03-1993
		ES 2091930 T	16-11-1996
		GR 3021974 T	31-03-1997
		US 5935809 A	10-08-1999
		US 5883076 A	16-03-1999
		US 5378819 A	03-01-1995
WO 9839422 A	11-09-1998	AU 6678098 A	22-09-1998
		BR 9808161 A	28-03-2000
		EP 0981605 A	01-03-2000

PATENT COOPERATION TREATY

M. Matlack

by fax and post

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

KENING LI
E.I. du Pont de Nemours and Company
Legal Patent Records Center
1007 Market Street
Wilmington, DE 19898
ETATS-UNIS D'AMERIQUE

FAX: 001-302 773 0164

PCT

SEP 12 2001

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

RECEIVED

Date of mailing *(day/month/year)*	10.09.2001

SEP 07 2001

PATENT RECORDS CENTER

Applicant's or agent's file reference	IMPORTANT NOTIFICATION
BB1356 PCT	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/11956	03/05/2000	07/05/1999

Applicant
E.I. DU PONT DE NEMOURS AND COMPANY

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Faux, K Tel.+49 89 2399-8062

REY NOTED

Form PCT/IPEA/416 (July 1992)

07 NO 2001

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
BB1356 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/11956	03/05/2000	07/05/1999
International Patent Classification (IPC) or national classification and IPC		
C12N15/82		
Applicant		
E.I. DU PONT DE NEMOURS AND COMPANY		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20/11/2000	10.09.01
Name and mailing address of the international preliminary examining authority:	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Keller, Y Telephone No. +49 89 2399 7419

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/11956

I. **Basis of the report**

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-30 as originally filed

Claims, No.:

21-24 as originally filed

1-20 filed with the demand

Sequence listing part of the description, pages:

1-40, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequenc listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT International application No. PCT/US00/11956

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under Item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-23 part and 24.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 1-23 part and 24.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/11956

Novelty (N)	Yes:	Claims	10-14, 20
	No:	Claims	1-9, 15-19
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-20
Industrial applicability (IA)	Yes:	Claims	1-20
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/US00/11956

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: EMBL, Acc: Q161346
D2: EMBL, Acc: AW061660
D3: EMBL, Acc: AW54624
D4: EMBL, Acc: AF036340
D5: Iris A. M., et al, The Plant cell, 1998, vol. 10 pages 2103-2113

1. The priority is valid only for sequences 1, 2 and related subject matter. Seq ID No 15-18 and related subject-matter are not covered by the priority documents, thus intermediate documents D2 and D3 are part of the prior art for the aforementioned subject-matter.

D1 discloses a DNA sequence from rice showing 83% identity (over 507 nucleotides) with SEQ ID No 17 and encoding a protein having 85% identity (over 165 amino acid) with SEQ ID No 18.

D2 discloses a cDNA sequence from maize showing 98% identity (over 596 nucleotides) with SEQ ID No 17 and encoding a protein having 96% identity (over 198 amino acid) with SEQ ID No 18.

D3 discloses a cDNA sequence from maize showing 99% identity (over 575 nucleotides) with the reverse complement of SEQ ID No 17 and encoding a protein having 100% identity (over 69 amino acid) with SEQ ID No 18.

D4 discloses the COI1 gene cDNA sequence from A. thaliana showing 57% identity (over 672 nucleotides) with SEQ ID No 1 and encoding a protein having 48% identity (over 196 amino acid) with SEQ ID No 2 and 57% identity (over 521 nucleotides) with SEQ ID No 15 and encoding a protein having 47% identity (over 186 amino acid) with SEQ ID No 16.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/US00/11956

D5 discloses that the COI1 gene is important in the plant response to pathogene attack (figure 2; discussion second paragraph) by intervening in the jasmonate response pathway.

1 In view of D2 claims 1-9, 15-19 do not meet the requirements of Art. 33(2) PCT.

2 The claimed nucleic acid are the result of BLAST searches (present application example), and their function relies solely on these results and thus are to be considered as a mere putative function.
As no function associated to the claimed nucleotide sequences could be unequivocally shown, the technical problem to be solved by the present application is to isolatea nucleic acid.
This does not involve an inventive step for the skilled person.
Therefore the claimed nucleic acids and related subject-matter (i.e claims 1-20) do not meet the requirements of Art. 33(3) PCT
Furthermore, it is common practice for the skilled person, when in presence of a nucleotide sequence encoding a protein (or parts thereof) with a given function, to isolate other nucleic acids encoding for proteins or polypeptides having related functions. These "related" nucleic acids can be isolated in standard techniques such as PCR, hybridisations, etc... with selected primers, oligonucleotides etc...
Furthermore said nucleic acids are routinely used for transforming organism (e.g plants). The use of ,non novel or non inventive nucleic acids, in techniques routinely used by the skilled person, does not involve from the man skilled in the art any inventive step.
Therefore, claims 10-14 and 20 do not meet the requirements of Art. 33(3) PCT.

Re Item VIII
Certain observations on the international application

Claim 16 is not clear since said claim is a dependent claim dependent from itself.

20.11.00

CLAIMS

What is claimed is:

1. An isolated polynucleotide that encodes a polypeptide of at least 60 amino acids, the polypeptide having a sequence identity of at least 95% based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs: 18, 20, 22, 24, 26, 28, 2, 4, 6, 8, 16, 30, 32, 34, 36, 10, 12, and 14.

2. The polynucleotide of Claim 1 wherein the polynucleotide encodes a polypeptide selected from the group consisting of SEQ ID NOs: 18, 20, 22, 24, 26, 28, 2, 4, 6, 8, 16, 30, 32, 34, 36, 10, 12, and 14.

3. The polynucleotide of Claim 1, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 17, 19, 21, 23, 25, 27, 1, 3, 5, 7, 15, 29, 31, 33, 35, 9, 11, and 13.

4. The polynucleotide of Claim 1, wherein the polypeptide is a disease resistance factor.

5. The polynucleotide of Claim 4, wherein the polypeptide is a COI1.

6. An isolated complement of the polynucleotide of Claim 1, wherein (a) the complement and the polynucleotide consist of the same number of nucleotides, and (b) the nucleotide sequences of the complement and the polynucleotide have 100% complementarity.

7. An isolated nucleic acid molecule that (1) comprises at least 180 nucleotides and (2) remains hybridized with the isolated polynucleotide of Claim 1 after a wash with 0.1X SSC, 0.1% SDS, and 65°C.

8. A cell comprising the polynucleotide of Claim 1.

9. The cell of Claim 8, wherein the cell is selected from the group consisting of a yeast cell, a bacterial cell and a plant cell.

10. A transgenic plant comprising the polynucleotide of Claim 1.

11. A method for transforming a cell comprising introducing into a cell the polynucleotide of Claim 1.

12. A method for producing a transgenic plant comprising (a) transforming a plant cell with the polynucleotide of Claim 1, and (b) regenerating a plant from the transformed plant cell.

13. A method for producing a polynucleotide fragment, the method comprising (a) selecting a nucleotide sequence comprised by the polynucleotide of Claim 1, and (b) producing a polynucleotide fragment containing the nucleotide sequence.

14. The method of Claim 13, wherein the fragment is produced *in vivo*.

15. An isolated polypeptide comprising (a) at least 60 amino acids, and (b) has a sequence identity of at least 95% based on the Clustal method compared to an amino acid sequence selected from the group consisting of SEQ ID NOs18, 20, 22, 24, 26, 28, 2, 4, 6, 8, 16, 30, 32, 34, 36, 10, 12, and 14.

16. The polypeptide of Claim 16 wherein the polypeptide has a sequence selected from the group consisting of SEQ ID NOs: 18, 20, 22, 24, 26, 28, 2, 4, 6, 8, 16, 30, 32, 34, 36, 10, 12, and 14.

17. The polypeptide of Claim 16, wherein the polypeptide is a disease resistance factor.

18. The polypeptide of Claim 17, wherein the polypeptide is a COI1.

19. A chimeric gene comprising the polynucleotide of Claim 1 operably linked to at least one suitable regulatory sequence.

20. A method for altering the level of disease resistance factor expression in a host cell, the method comprising:

(a) Transforming a host cell with the chimeric gene of claim 20; and
(b) Growing the transformed cell in step (a) under conditions suitable for the expression of the chimeric gene.

14

PATENT COOPERATION TREATY

PCT

REC'D 12 SEP 2001
WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
BB1356 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/11956	03/05/2000	07/05/1999
International Patent Classification (IPC) or national classification and IPC C12N15/82		
Applicant E.I. DU PONT DE NEMOURS AND COMPANY		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20/11/2000	10.09.01
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Keller, Y Telephone No. +49 89 2399 7419

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/11956

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-30 as originally filed

Claims, No.:

21-24 as originally filed

1-20 filed with the demand

Sequence listing part of the description, pages:

1-40, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 1-23 part and 24.

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-23 part and 24.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	10-14, 20
	No:	Claims	1-9, 15-19
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-20
Industrial applicability (IA)	Yes:	Claims	1-20
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: EMBL, Acc: Q161346
D2: EMBL, Acc: AW061660
D3: EMBL, Acc: AW54624
D4: EMBL, Acc: AF036340
D5: Iris A. M., et al, The Plant cell, 1998, vol. 10 pages 2103-2113

1. The priority is valid only for sequences 1, 2 and related subject matter. Seq ID No 15-18 and related subject-matter are not covered by the priority documents, thus intermediate documents D2 and D3 are part of the prior art for the aforementioned subject-matter.

D1 discloses a DNA sequence from rice showing 83% identity (over 507 nucleotides) with SEQ ID No 17 and encoding a protein having 85% identity (over 165 amino acid) with SEQ ID No 18.

D2 discloses a cDNA sequence from maize showing 98% identity (over 596 nucleotides) with SEQ ID No 17 and encoding a protein having 96% identity (over 198 amino acid) with SEQ ID No 18.

D3 discloses a cDNA sequence from maize showing 99% identity (over 575 nucleotides) with the reverse complement of SEQ ID No 17 and encoding a protein having 100% identity (over 69 amino acid) with SEQ ID No 18.

D4 discloses the COI1 gene cDNA sequence from A. thaliana showing 57% identity (over 672 nucleotides) with SEQ ID No 1 and encoding a protein having 48% identity (over 196 amino acid) with SEQ ID No 2 and 57% identity (over 521 nucleotides) with SEQ ID No 15 and encoding a protein having 47% identity (over 186 amino acid) with SEQ ID No 16.

D5 discloses that the COI1 gene is important in the plant response to pathogene attack (figure 2; discussion second paragraph) by intervening in the jasmonate response pathway.

1 In view of D2 claims 1-9, 15-19 do not meet the requirements of Art. 33(2) PCT.

2 The claimed nucleic acid are the result of BLAST searches (present application example), and their function relies solely on these results and thus are to be considered as a mere putative function.
As no function associated to the claimed nucleotide sequences could be unequivocally shown, the technical problem to be solved by the present application is to isolatea nucleic acid.
This does not involve an inventive step for the skilled person.
Therefore the claimed nucleic acids and related subject-matter (i.e claims 1-20) do not meet the requirements of Art. 33(3) PCT
Furthermore, it is common practice for the skilled person, when in presence of a nucleotide sequence encoding a protein (or parts thereof) with a given function, to isolate other nucleic acids encoding for proteins or polypeptides having related functions. These "related" nucleic acids can be isolated in standard techniques such as PCR, hybridisations, etc... with selected primers, oligonucleotides etc...
Furthermore said nucleic acids are routinely used for transforming organism (e.g plants). The use of ,non novel or non inventive nucleic acids, in techniques routinely used by the skilled person, does not involve from the man skilled in the art any inventive step.
Therefore, claims 10-14 and 20 do not meet the requirements of Art. 33(3) PCT.

Re Item VIII
Certain observations on the international application

Claim 16 is not clear since said claim is a dependent claim dependent from itself.

CLAIMS

What is claimed is:

1. An isolated polynucleotide that encodes a polypeptide of at least 60 amino acids, the polypeptide having a sequence identity of at least 95% based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs: 18, 20, 22, 24, 26, 28, 2, 4, 6, 8, 16, 30, 32, 34, 36, 10, 12, and 14.

2. The polynucleotide of Claim 1 wherein the polynucleotide encodes a polypeptide selected from the group consisting of SEQ ID NOs: 18, 20, 22, 24, 26, 28, 2, 4, 6, 8, 16, 30, 32, 34, 36, 10, 12, and 14.

3. The polynucleotide of Claim 1, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 17, 19, 21, 23, 25, 27, 1, 3, 5, 7, 15, 29, 31, 33, 35, 9, 11, and 13.

4. The polynucleotide of Claim 1, wherein the polypeptide is a disease resistance factor.

5. The polynucleotide of Claim 4, wherein the polypeptide is a COI1.

6. An isolated complement of the polynucleotide of Claim 1, wherein (a) the complement and the polynucleotide consist of the same number of nucleotides, and (b) the nucleotide sequences of the complement and the polynucleotide have 100% complementarity.

7. An isolated nucleic acid molecule that (1) comprises at least 180 nucleotides and (2) remains hybridized with the isolated polynucleotide of Claim 1 after a wash with 0.1X SSC, 0.1% SDS, and 65°C.

8. A cell comprising the polynucleotide of Claim 1.

9. The cell of Claim 8, wherein the cell is selected from the group consisting of a yeast cell, a bacterial cell and a plant cell.

10. A transgenic plant comprising the polynucleotide of Claim 1.

11. A method for transforming a cell comprising introducing into a cell the polynucleotide of Claim 1.

12. A method for producing a transgenic plant comprising (a) transforming a plant cell with the polynucleotide of Claim 1, and (b) regenerating a plant from the transformed plant cell.

13. A method for producing a polynucleotide fragment, the method comprising (a) selecting a nucleotide sequence comprised by the polynucleotide of Claim 1, and (b) producing a polynucleotide fragment containing the nucleotide sequence.

14. The method of Claim 13, wherein the fragment is produced *in vivo*.

15. An isolated polypeptide comprising (a) at least 60 amino acids, and (b) has a sequence identity of at least 95% based on the Clustal method compared to an amino acid sequence selected from the group consisting of SEQ ID NOs18, 20, 22, 24, 26, 28, 2, 4, 6, 8, 16, 30, 32, 34, 36, 10, 12, and 14.

16. The polypeptide of Claim 16 wherein the polypeptide has a sequence selected from the group consisting of SEQ ID NOs: 18, 20, 22, 24, 26, 28, 2, 4, 6, 8, 16, 30, 32, 34, 36, 10, 12, and 14.

17. The polypeptide of Claim 16, wherein the polypeptide is a disease resistance factor.

18. The polypeptide of Claim 17, wherein the polypeptide is a COI1.

19. A chimeric gene comprising the polynucleotide of Claim 1 operably linked to at least one suitable regulatory sequence.

20. A method for altering the level of disease resistance factor expression in a host cell, the method comprising:

(a) Transforming a host cell with the chimeric gene of claim 20; and
(b) Growing the transformed cell in step (a) under conditions suitable for the expression of the chimeric gene.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 13 December 2000 (13.12.00)	
International application No. PCT/US00/11956	**Applicant's or agent's file reference** BB1356 PCT
International filing date (day/month/year) 03 May 2000 (03.05.00)	**Priority date** (day/month/year) 07 May 1999 (07.05.99)
Applicant CAIMI, Perry, G. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

20 November 2000 (20.11.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Zakaria EL KHODARY
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

KL 66 BB1356

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

APR 23 2001

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 November 2000 (16.11.2000)

PCT

(10) International Publication Number
WO 00/68406 A3

(51) International Patent Classification[7]: C12N 15/82, 15/29, C07K 14/415, C12Q 1/68

(21) International Application Number: PCT/US00/11956

(22) International Filing Date: 3 May 2000 (03.05.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/133,041 7 May 1999 (07.05.1999) US

(71) Applicant *(for all designated States except US)*: **E.I. DU PONT DE NEMOURS AND COMPANY** [US/US]; 1007 Market Street, Wilmington, DE 19898 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **CAIMI, Perry, G.** [US/US]; 7 Holly Drive, Kennett Square, PA 19348 (US). **FAMODU, Omolayo, O.** [US/US]; 216 Barrett Run Place, Newark, DE 19702 (US). **LEE, Jiang-Ming** [CN/US]; 13 Pine Tree Place, West Caldwell, NJ 07006 (US). **MIAO, Guo-Hua** [US/US]; 202 Cheery Blossom Place, Hockessin, DE 19707 (US). **MAXWELL, Carl, A.** [US/US]; 35 Mary Anita Court, Elton, MD 21921 (US).

(74) Agent: **GEIGER, Kathleen, W.**; E.I. du Pont de Nemours and Company, Legal Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

(88) Date of publication of the international search report:
12 April 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

RECEIVED
APR 23 2001
PATENT RECORDS CENTER

WO 00/68406 A3

(54) Title: DISEASE RESISTANCE FACTORS

(57) Abstract: This invention relates to an isolated nucleic acid fragment encoding a disease resistance factor. The invention also relates to the construction of a chimeric gene encoding all or a substantial portion of the disease resistance factor, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the disease resistance factor in a transformed host cell.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12N 15/82, 15/29, C07K 14/415, C12Q 1/68 — A2

(11) International Publication Number: WO 00/68406

(43) International Publication Date: 16 November 2000 (16.11.00)

(21) International Application Number: PCT/US00/11956

(22) International Filing Date: 3 May 2000 (03.05.00)

(30) Priority Data:
60/133,041 7 May 1999 (07.05.99) US

(71) Applicant *(for all designated States except US)*: E.I. DU PONT DE NEMOURS AND COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: CAIMI, Perry, G. [US/US]; 7 Holly Drive, Kennett Square, PA 19348 (US). FAMODU, Omolayo, O. [US/US]; 216 Barrett Run Place, Newark, DE 19702 (US). LEE, Jiang–Ming [CN/US]; 13 Pine Tree Place, West Caldwell, NJ 07006 (US). MIAO, Guo–Hua [US/US]; 202 Cheery Blossom Place, Hockessin, DE 19707 (US). MAXWELL, Carl, A. [US/US]; 35 Mary Anita Court, Elton, MD 21921 (US).

(74) Agent: GEIGER, Kathleen, W.; E.I. du Pont de Nemours and Company, Legal Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: DISEASE RESISTANCE FACTORS

```
SEQ ID NO:18  ------------------------------------------------------------
SEQ ID NO:22  TKTSAPFLFTLSLRSNMTEERNVRKTRV-------VDVVLDCVIPYIDDPKDRDAVSQVC
SEQ ID NO:20  MGGEAP------------EARRLDRAMSFGGAGSIPEEALHLVLGYVDDPRDREAVSLVC
SEQ ID NO:37  M-----------------EDPDIKRCKL--SCVATVDDVIEQVMTYITDPKDRDSASLVC
                1                                                       60

SEQ ID NO:18  ----------------------------------------TRPRT--------------
SEQ ID NO:22  RRWYELDSLTRKHVTIALCYTTTPARLRRRFPHLESLKLKGKPRAAMFNLIPEDWGGHVT
SEQ ID NO:20  RRWHRIDALTRKHVTVPFCYAASPAHLLARFPRLESLAVKGKPRAAMYGLIPEDWGAYAR
SEQ ID NO:37  RRWFKIDSETREHVTMALCYTATPDRLSRRFPNLRSLKLKGKPRAAMFNLIPENWGGYVT
               61                                                      120

SEQ ID NO:18  ------------------------------------------------------------
SEQ ID NO:22  PWVKEISQYFDCLKSLHFRRMIVKDSDLQNLARDRGHVLHALKLDKCSGFTTDGLFHIGR
SEQ ID NO:20  PWVAELAAPLECLKALHLRRMVVTDDDLAALVRARGHMLQELKLDKCSGFSTDALRLVAR
SEQ ID NO:37  PWVTEISNNLRQLKSVHFRRMIVSDLDLDRLAKARADDLETLKLDKCSGFTTDGLLSIVT
              121                                                      180

                       *  **          *   **  *  ** ***  *          **     *
SEQ ID NO:18  --RGLETLFLEESTIDEKENDEWIRELATSNSVLETLNFFLTDL-RASPEYLTLLVRNCQ
SEQ ID NO:22  FCKSLRVLFLEESSILEKD-GEWLHELALNNTVLETLNFYLTDIAVVKIEDLELLAKNCP
SEQ ID NO:20  SCRSLRTLFLEECSIADNGT-EWLHDLAVNNPVLETLNFHMTEL-TVVPADLELLAKKCK
SEQ ID NO:37  HCRKIKTLLMEESSFSEKD-GKWLHELAQHNTSLEVLNFYMTEFAKISPKDLETIARNCR
              181                                                      240
```

(57) Abstract

This invention relates to an isolated nucleic acid fragment encoding a disease resistance factor. The invention also relates to the construction of a chimeric gene encoding all or a substantial portion of the disease resistance factor, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the disease resistance factor in a transformed host cell.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

INTERNATIONAL SEARCH REPORT

Int' tional Application No
PCT/US 00/11956

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C12N15/29 C07K14/415 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EMBL, BIOSIS, EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL [Online] ACCESSION NO:AQ161346, 9 September 1998 (1998-09-09) WING R.A.: "nbxb0006F06f CUGI Rice BAC Library Oryza sativa genomic clone nbxb0006F06f, genomic survey sequence." XP002146944 see sequence ---	1-4,16, 18
P,X	DATABASE EMBL [Online] ACCESSION NO:AW061660, 6 October 1999 (1999-10-06) WALBOT V.: "660012G08.y1 660 - Mixed stages of anther and pollen Zea mays cDNA, mRNA sequence" XP002146880 see sequence --- -/--	1-4,16, 18

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 September 2000	22 12 2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	MADDOX, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	DATABASE EMBL [Online] ACCESSION NO:AW054624, 26 September 1999 (1999-09-26) WALBOT V.: "660012G08.x1 660 - Mixed stages of anther and pollen Zea mays cDNA, mRNA sequence." XP002146945 see sequence --- DATABASE EMBL [Online] ACCESSION NO:AF036340, 29 May 1998 (1998-05-29) FEYS B.J., ET AL.: "Arabidopsis thaliana LRR-containing F-box protein (COI1) mRNA, complete cds." XP002146881 see sequence -& XIE, D.-X., ET AL.: "COI1: An Arabidsopsis gene required for jasmonate-regulated defense and fertility" SCIENCE, vol. 280, 15 May 1998 (1998-05-15), pages 1091-1094, XP002146875 the whole document -& FEYS, B.J., ET AL.: "ARABIDOPSIS MUTANTS SELECTED FOR RESISTANCE TO THE PHYTOTOXIN CORONATINE ARE MALE STERILE, INSENSITIVE TO METHYL JASMONATE AND RESISTANT TO A BACTERIAL PATHOGEN" THE PLANT CELL, vol. 6, 1994, pages 751-759, XP002049621 the whole document ---	1-4,16, 18 1-23
A	DATABASE EMBL [Online] ACCESSION NO:AI444738, 16 March 1999 (1999-03-16) WALBOT, V.: "486015G10.x5 486 - leaf primordia cDNA library from Hake lab Zea mays cDNA, mRNA sequence" XP002146974 see sequence --- DATABASE EMBL [Online] ACCESSION NO:AU032235, 19 October 1998 (1998-10-19) SASKAI, T.: "Oryza sativa cDNA, partial sequence (R3783_1A)." XP002146975 see sequence --- -/--	1-10 1-10

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BENEDETTI CELSO E ET AL: "Differential expression of a novel gene in response to coronatine, methyl jasmonate, and wounding in the Coil mutant of arabidopsis." PLANT PHYSIOLOGY (ROCKVILLE), vol. 116, no. 3, March 1998 (1998-03), pages 1037-1042, XP002146876 ISSN: 0032-0889 the whole document	1-23
A	WO 98 00023 A (KAZAN KEMAL ;MANNERS JOHN MICHAEL (AU); BROEKAERT WILLEM FRANS (BE) 8 January 1998 (1998-01-08) claim 7	23
A	WO 91 18512 A (UNIV WASHINGTON) 12 December 1991 (1991-12-12) the whole document	23
A	PENNINCKX IRIS A M A ET AL: "Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in arabidopsis." PLANT CELL, vol. 10, no. 12, December 1998 (1998-12), pages 2103-2113, XP002146877 ISSN: 1040-4651 the whole document	23
A	THOMMA BART P H J ET AL: "Separate jasmonate-dependent and salicylate-dependent defense-response pathways in Arabidopsis are essential for resistance to distinct microbial pathogens." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 95, no. 25, December 1998 (1998-12), pages 15107-15111, XP002146878 Dec., 1998 ISSN: 0027-8424 the whole document	23
A	CORDERO MARIA JOSE ET AL: "Expression of a maize proteinase inhibitor gene is induced in response to wounding and fungal infection: Systemic wound-response of a monocot gene." PLANT JOURNAL, vol. 6, no. 2, 1994, pages 141-150, XP002146879 ISSN: 0960-7412	

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	GRAY J ET AL: "A NOVEL SUPPRESSOR OF CELL DEATH IN PLANTS ENCODED BY THE LLS 1 GENE OF MAIZE" CELL,US,CELL PRESS, CAMBRIDGE, NA, vol. 89, 4 April 1997 (1997-04-04), pages 25-31, XP002068010 ISSN: 0092-8674 -& DATABASE EMBL [Online] ACCESSION NO:U77346, 18 April 1997 (1997-04-18) GRAY, J. ET AL.: "Zea mays lethal leaf-spot 1 (lls1) gene, partial cds." XP002068011 -& DATABASE EMBL [Online] ACCESSION NO:U77345, 18 April 1997 (1997-04-18) GRAY, J. ET AL.: "Zea mays lethal leaf-spot 1 (lls1) mRNA, partial cds." XP002146987 abstract --- WO 98 39422 A (GRAY JOHN ;PIONEER HI BRED INT (US); UNIV MISSOURI (US); BRIGGS ST) 11 September 1998 (1998-09-11) -----	

INTERNATIONAL SEARCH REPORT

...ernational application No.
PCT/US 00/11956

Box I Observations where certain claims were found unsearchable (Continuation of it m 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-23 all partially

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-23 all partially

Polynucleotides from corn encoding COI1 polypeptides,and COI1 polypeptides as specified in SEQ ID NOS:1,2,and 15-18,chimeric genes, methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection based on said sequences.

2. Claims: 1-23 all partially

Polynucleotides from rice encoding COI1 polypeptides,and COI1 polypeptides as specified in SEQ ID NOS:3,4,19 and 20,chimeric genes,methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection based on said sequences.

3. Claims: 1-23 all partially

Polynucleotides from soybean encoding COI1 polypeptides,and COI1 polypeptides as specified in SEQ ID NOS:5,6,21 and 22,chimeric genes, methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection based on said sequences.

4. Claims: 1-23 all partially

Polynucleotides from wheat encoding COI1 polypeptides,and COI1 polypeptides as specified in SEQ ID NOS:7,8,and 23-28,chimeric genes, methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection based on said sequences.

5. Claims: 1-24 all partially

Polynucleotides from rice encoding Lls1 polypeptides,and Lls1 polypeptides as specified in SEQ ID NOS: 9,10,29 and 30,chimeric genes, methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection, and method for evaluating the ability of a compound to inhibit Lls1, based on said sequences.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

6. Claims: 1-24 all partially

Polynucleotides from soybean encoding Lls1 polypeptides,and Lls1 polypeptides as specified in SEQ ID NOS:11,12,31 and 32,chimeric genes, methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection, and method for evaluating the ability of a compound to inhibit Lls1, based on said sequences.

7. Claims: 1-24 all partially

Polynucleotides from wheat encoding Lls1 polypeptides,and Lls1 polypeptides as specified in SEQ ID NOS:13, 14,33-36,chimeric genes, methods for selecting isolated polynucleotides, and obtaining nucleic acid fragments encoding disease resistance factors, compositions and methods for positive selection, and method for evaluating the ability of a compound to inhibit Lls1, based on said sequences.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int tional Application No
PCT/US 00/11956

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9800023 A	08-01-1998	AU 3183597 A	21-01-1998
		BR 9710000 A	10-08-1999
		EP 0912096 A	06-05-1999
WO 9118512 A	12-12-1991	AT 142420 T	15-09-1996
		AU 650459 B	23-06-1994
		AU 7953191 A	31-12-1991
		CA 2083595 A	26-11-1991
		DE 69122100 D	17-10-1996
		DE 69122100 T	06-02-1997
		DK 532650 T	07-10-1996
		EP 0532650 A	24-03-1993
		ES 2091930 T	16-11-1996
		GR 3021974 T	31-03-1997
		US 5935809 A	10-08-1999
		US 5883076 A	16-03-1999
		US 5378819 A	03-01-1995
WO 9839422 A	11-09-1998	AU 6678098 A	22-09-1998
		BR 9808161 A	28-03-2000
		EP 0981605 A	01-03-2000

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
BB1356 PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 00/ 11956	03/05/2000	07/05/1999
Applicant		
E.I. DU PONT DE NEMOURS AND COMPANY		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 8 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   ☒ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readble form.

   ☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

   ☐ as suggested by the applicant.

   ☐ because the applicant failed to suggest a figure.

   ☐ because this figure better characterizes the invention.

   ☒ None of the figures.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In. ational Application No
PCT/US 98/04040

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9703183 A	30-01-1997	US 5756322 A	26-05-1998
		AU 6488696 A	10-02-1997
		EP 0840782 A	13-05-1998
WO 9535318 A	28-12-1995	US 5650553 A	22-07-1997
		AU 686408 B	05-02-1998
		AU 2865095 A	15-01-1996
		CA 2193255 A	28-12-1995
		EP 0763060 A	19-03-1997
WO 9804586 A	05-02-1998	AU 3702897 A	20-02-1998

BNSDOCID: <WO___9839422A1_I_>

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/US 98/04040

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N9/02 C12N15/82 C12N5/10 C12Q1/68 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12Q A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DANGL, J. L., ET AL: "DEATH DON'T HAVE NO MERCY: CELL DEATH PROGRAMS IN PLANT-MICROBE INTERACTIONS" PLANT CELL, vol. 8, no. 10, October 1996, pages 1793-1807, XP002035757 pages 1795, right column, page 1796, left column, page 1799 --- -/--	1-11,50, 51

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 June 1998	03.07.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Holtorf, S

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int ational Application No
PCT/US 98/04040

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOHAL, G.S., ET AL. : "a tale of two mimics: transposon mutagenesis and characterization of two disease lesion mimic mutations of maize" MAYDICA, vol. 39, 1994, pages 69-76, XP002068008 cited in the application page 69, right column, page 70, page 75, especially right column, last paragraph ---	6-11,50, 51
X	WO 97 03183 A (UNIV RUTGERS ;TUMER NILGUN E (US)) 30 January 1997 page 1; pages 4, especially line 9-11; ---	1,12,18, 19, 25-27, 29,30,50
A	JOHAL, G.S., ET AL.: "DISEASE LESION MIMICS OF MAIZE: A MODEL FOR CELL DEATH IN PLANTS" BIOESSAYS, vol. 17, no. 8, 1995, pages 685-692, XP002068009 cited in the application last paragraph see page 690, right-hand column ---	1-57
A	WO 95 35318 A (UNIV PENNSYLVANIA) 28 December 1995 see page 15, line 25 - line 36 ---	1-57
P,X	GRAY,J., ET AL. : "a novel suppressor of cell death in plants encoded by the lls1 gene of maize" CELL, vol. 89, 4 April 1997, pages 25-31, XP002068010 see the whole document ---	1-11,50
P,X	WO 98 04586 A (INNES JOHN CENTRE INNOV LTD ;PANSTRUGA RALPH (GB); BUESCHGES RAINE) 5 February 1998 page 1, line 10-13; page 2, line 7-17; page 5,35,36; page 37, line 16-21; page 47, line 21-25; page 49, line 10-17 ---	1,12,14, 18,19, 21, 25-27, 29,50
P,X	GRAY, J., ET AL. : "a novel suppressor of cell death in plants encoded by the lls1 gene of maize" EMBL SEQUENCE DATA LIBRARY, 18 April 1997, HEIDELBERG, GERMANY, XP002068011 accession no. U77346 ---	37,38
1	-/--	

INTERNATIONAL SEARCH REPORT

Int .tional Application No
PCT/US 98/04040

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	NEWMAN, T. ,ET AL.: "untitled" EMBL SEQUENCE DATA LIBRARY, 1 July 1997, HEIDELBERG, GERMANY, XP002068012 accession no. 004422 ---	1-3
P,X	NEWMAN T., ET AL. : "genes galore: a summary of methods for assessing results from large-scale partial sequencing of anonymous arabidosis cDNA clones" EMBL SEQUENCE DATA LIBRARY, 10 June 1997, HEIDELBERG, GERMANY, XP002068013 accession no. U77347 ---	10,50
T	CALIEBE, A., ET AL.: "the chloroplastic protein import machinery contains a rieske-type iron-sulfur cluster and a mononuclear iron-binding protein" THE EMBO JOURNAL , vol. 16, no. 24, 15 December 1997, pages 7342-7350, XP002068014 pages 7348, last paragraph, page 7344, right column, Fig. 3b -----	1-57

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/04040

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

Claim 26 was searched as if it is referring to the method of claim 25.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In. ational Application No
PCT/US 98/04040

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9703183 A	30-01-1997	US 5756322 A	26-05-1998
		AU 6488696 A	10-02-1997
		EP 0840782 A	13-05-1998
WO 9535318 A	28-12-1995	US 5650553 A	22-07-1997
		AU 686408 B	05-02-1998
		AU 2865095 A	15-01-1996
		CA 2193255 A	28-12-1995
		EP 0763060 A	19-03-1997
WO 9804586 A	05-02-1998	AU 3702897 A	20-02-1998

TITLE

DISEASE RESISTANCE FACTORS

This application claims the benefit of U.S. Provisional Application No. 60/133,041, filed May 7, 1999.

FIELD OF THE INVENTION

This invention is in the field of plant molecular biology. More specifically, this invention pertains to nucleic acid fragments encoding disease resistance factors in plants and seeds.

BACKGROUND OF THE INVENTION

Plants synthesize signaling molecules in response to wounding, herbivore attack and pathogen attack. These compounds are derived from linoleic acid and stimulate the expression of several genes referred to as jasmonate-induced proteins. These include proteinase inhibitors, thionins, vegetative storage proteins, lipoxygenases, ribosome-inactivating proteins, enzymes of phenylpropanoid metabolism, and others. The jasmonates can also repress the expression of genes related to photosynthesis at the transcriptional and translational levels.

Coronatine is a phytotoxin produced by several pathovars of *Pseudomonas syringae* which induces leaf chlorosis, inhibits root growth and is thought to play a role in disease development by suppressing the disease-resistance genes and mimicking the action of methyl jasmonate. An *Arabidopsis thaliana* gene induced by coronatine, methyl jasmonate, and wounding has been characterized and is known by two different names: ATHCOR1 (for *A. thaliana* coronatine-induced) and COI1. ATHCOR1 has been shown to be expressed in seedlings, mature leaves and flowers and to contain conserved amino acid sequence domains present in bacterial, plant and animal hydrolases (Benedetti et al. (1998) *Plant Physiol. 116*:1037-1042). The COI1 protein contains an F-box-like motif and leucine-rich repeats. This protein may recruit regulators of defense response and pollen development for modification by ubiquitination (Xie et al. (1998) *Science 280*:1091-1094).

The maize Lls1 (lethal leaf spot1) locus is characterized by the initiation of necrotic lesions which expand to kill leaf-cells autonomously. The Lls1 gene is required to limit the spread of cell death in mature leaves. The Lls1-encoded protein (LLS1) contains two consensus binding motifs of aromatic ring-hydroxylating dioxygenases and may function to degrade a phenolic mediator of cell death. The LLS1 protein is expressed predominantly in the leaf epidermal tissue (Simmons et al. (1998) *Mol. Plant Microbe Interact. 11*:1110-1118; Gray et al. (1997) *Cell 89*:25-31).

The sequences encoding COI1 from corn, rice, soybean and wheat have yet to be determined as are the sequences encoding rice, soybean and wheat LLS1. Manipulation of the COI1 or LLS1 genes will be useful in engineering broad spectrum disease, insect and

stress resistance. The genes encoding LLS1 will also be useful for herbicide discovery and design.

SUMMARY OF THE INVENTION

The present invention concerns an isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of: (a) a first nucleotide sequence encoding a polypeptide of at least 60 amino acids selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36; or (b) a second nucleotide sequence comprising the complement of the first nucleotide sequence.

In a second embodiment, it is preferred that the isolated polynucleotide of the claimed invention comprises a first nucleotide sequence which comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, and 35.

In a third embodiment, this invention concerns an isolated polynucleotide comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, and 35 and the complement of such nucleotide sequences.

In a fourth embodiment, this invention relates to a chimeric gene comprising an isolated polynucleotide of the present invention operably linked to at least one suitable regulatory sequence.

In a fifth embodiment, the present invention concerns a host cell comprising a chimeric gene of the present invention or an isolated polynucleotide of the present invention. The host cell may be eukaryotic, such as a yeast or a plant cell, or prokaryotic, such as a bacterial cell. The present invention also relates to a virus, preferably a baculovirus, comprising an isolated polynucleotide of the present invention or a chimeric gene of the present invention.

In a sixth embodiment, the invention also relates to a process for producing a host cell comprising a chimeric gene of the present invention or an isolated polynucleotide of the present invention, the process comprising either transforming or transfecting a compatible host cell with a chimeric gene or isolated polynucleotide of the present invention.

In a seventh embodiment, the invention concerns a COI1 or an LLS1 protein of at least 60 amino acids comprising a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36.

In an eighth embodiment, the invention relates to a method of selecting an isolated polynucleotide that affects the level of expression of a COI1 or an LLS1 protein or enzyme activity in a host cell, preferably a plant cell, the method comprising the steps of:
(a) constructing an isolated polynucleotide of the present invention or a chimeric gene of the present invention; (b) introducing the isolated polynucleotide or the chimeric gene into a host cell; (c) measuring the level of the COI1 or the LLS1 protein or enzyme activity in the

host cell containing the isolated polynucleotide; and (d) comparing the level of the COI1 or the LLS1 protein or enzyme activity in the host cell containing the isolated polynucleotide with the level of the COI1 or the LLS1 protein or enzyme activity in the host cell that does not contain the isolated polynucleotide.

In a ninth embodiment, the invention concerns a method of obtaining a nucleic acid fragment encoding a substantial portion of a COI1 or an LLS1 protein, preferably a plant COI1 or LLS1 protein, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, and 35, and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a substantial portion of a COI1 or an LLS1 protein amino acid sequence.

In a tenth embodiment, this invention relates to a method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding a COI1 or an LLS1 protein comprising the steps of: probing a cDNA or genomic library with an isolated polynucleotide of the present invention; identifying a DNA clone that hybridizes with an isolated polynucleotide of the present invention; isolating the identified DNA clone; and sequencing the cDNA or genomic fragment that comprises the isolated DNA clone.

In an eleventh embodiment, this invention concerns a composition, such as a hybridization mixture, comprising an isolated polynucleotide or an isolated polypeptide of the present invention.

In a twelfth embodiment, this invention concerns a method for positive selection of a transformed cell comprising: (a) transforming a host cell with the chimeric gene of the present invention or a construct of the present invention; and (b) growing the transformed host cell, preferably a plant cell, such as a monocot or a dicot, under conditions which allow expression of the COI1 or the LLS1 protein polynucleotide in an amount sufficient to complement a null mutant to provide a positive selection means.

In a thirteenth embodiment, this invention relates to a method of altering the level of expression of a disease resistance factor in a host cell comprising: (a) transforming a host cell with a chimeric gene of the present invention; and (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of altered levels of the disease resistance factor in the transformed host cell.

A further embodiment of the instant invention is a method for evaluating at least one compound for its ability to inhibit the activity of an LLS1 protein, the method comprising the steps of: (a) transforming a host cell with a chimeric gene comprising a nucleic acid

fragment encoding an LLS1 polypeptide, operably linked to at least one suitable regulatory sequence; (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of LLS1 protein in the transformed host cell; (c) optionally purifying the LLS1 protein expressed by the transformed host cell; (d) treating the LLS1 protein with a compound to be tested; and (e) comparing the activity of the LLS1 protein that has been treated with a test compound to the activity of an untreated LLS1 protein, thereby selecting compounds with potential for inhibitory activity.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE LISTING

The invention can be more fully understood from the following detailed description, the accompanying drawings and the Sequence Listing which form a part of this application.

Figure 1 presents an alignment of the amino acid sequences derived from corn clone p0128.cpici34r:fis (SEQ ID NO:18), rice clone rl0n.pk099.p14:fis (SEQ ID NO:20), and soybean clone sgs4c.pk003.k23:fis (SEQ ID NO:22) with the *Arabidopsis thaliana* COI1 sequence (NCBI General Identifier No. 3158394; SEQ ID NO:37). Underlined amino acids in SEQ ID NO:37 correspond to the degenerate F-box motif and the 16 imperfect leucine-rich repeats (LRRs) indicated by Xie et al. (1998, *Science 280*:1091-1094). Amino acids conserved among all the species are indicated by an Asterisk (*) above the alignment. Dashes are used by the program to maximize the alignment.

Figure 2 presents an alignment of the amino acid sequences derived from rice clone rds2c.pk005.b12:fis (SEQ ID NO:30), soybean clone sgc2c.pk001.c22:fis (SEQ ID NO:32), and wheat clone wlmk1.pk0015.h3:fis (SEQ ID NO:36) with the *Zea mays* LLS1 sequence (NCBI General Identifier Nos. 7489721, SEQ ID NO:38). Underlined amino acids in SEQ ID NO:38 correspond to consensus sequence for coordinating the Reiske-type [2Fe-2S] cluster and the mononuclear non-heme binding site (Gray et al. (1997) *Cell 89*:25-31). Amino acids conserved among all sequences are indicated by an Asterisk (*) above the alignment. Dashes are used by the program to maximize the alignment.

Table 1 lists the polypeptides that are described herein, the designation of the cDNA clones that comprise the nucleic acid fragments encoding polypeptides representing all or a substantial portion of these polypeptides, and the corresponding identifier (SEQ ID NO:) as used in the attached Sequence Listing. The sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

TABLE 1

Disease Resistance Factors

Protein	Clone Designation	SEQ ID NO: (Nucleotide)	SEQ ID NO: (Amino Acid)
Corn COI1	p0128.cpici34r	1	2
Rice COI1	Contig of: rlr2.pk0027.h4 rl0n.pk099.p14 rl0n.pk0047.c5	3	4
Soybean COI1	sgs4c.pk003.k23	5	6
Wheat COI1	Contig of: wre1n.pk0122.d3 wl1n.pk0018.f8	7	8
Rice LLS1	rds2c.pk005.b12	9	10
Soybean LLS1	sgc2c.pk001.c22	11	12
Wheat LLS1	wlmk1.pk0015.h3	13	14
Corn COI1	p0037.crwbs69r	15	16
Corn COI1	p0128.cpici34r:fis	17	18
Rice COI1	rl0n.pk099.p14:fis	19	20
Soybean COI1	sgs4c.pk003.k23:fis	21	22
Wheat COI1	wl1n.pk0049.f7	23	24
Wheat COI1	wlm0.pk0009.d7	25	26
Wheat COI1	wre1n.pk0122.d3:fis	27	28
Rice LLS1	rds2c.pk005.b12:fis	29	30
Soybean LLS1	sgc2c.pk001.c22:fis	31	32
Wheat LLS1	wlm0.pk0002.c10	33	34
Wheat LLS1	wlmk1.pk0015.h3:fis	35	36

The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in *Nucleic Acids Res. 13*:3021-3030 (1985) and in the *Biochemical J. 219 (No. 2)*:345-373 (1984) which are herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

DETAILED DESCRIPTION OF THE INVENTION

In the context of this disclosure, a number of terms shall be utilized. The terms "polynucleotide", "polynucleotide sequence", "nucleic acid sequence", and "nucleic acid fragment"/"isolated nucleic acid fragment" are used interchangeably herein. These terms encompass nucleotide sequences and the like. A polynucleotide may be a polymer of RNA

or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. An isolated polynucleotide of the present invention may include at least one of 60 contiguous nucleotides, preferably at least one of 40 contiguous nucleotides, most preferably one of at least 30 contiguous nucleotides derived from SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, and 35, or the complement of such sequences.

The term "isolated polynucleotide" refers to a polynucleotide that is substantially free from other nucleic acid sequences, such as and not limited to other chromosomal and extrachromosomal DNA and RNA. Isolated polynucleotides may be purified from a host cell in which they naturally occur. Conventional nucleic acid purification methods known to skilled artisans may be used to obtain isolated polynucleotides. The term also embraces recombinant polynucleotides and chemically synthesized polynucleotides.

The term "recombinant" means, for example, that a nucleic acid sequence is made by an artificial combination of two otherwise separated segments of sequence, e.g., by chemical synthesis or by the manipulation of isolated nucleic acids by genetic engineering techniques.

As used herein, "contig" refers to a nucleotide sequence that is assembled from two or more constituent nucleotide sequences that share common or overlapping regions of sequence homology. For example, the nucleotide sequences of two or more nucleic acid fragments can be compared and aligned in order to identify common or overlapping sequences. Where common or overlapping sequences exist between two or more nucleic acid fragments, the sequences (and thus their corresponding nucleic acid fragments) can be assembled into a single contiguous nucleotide sequence.

As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the polypeptide encoded by the nucleotide sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by gene silencing through for example antisense or co-suppression technology. "Substantially similar" also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially affect the functional properties of the resulting transcript vis-à-vis the ability to mediate gene silencing or alteration of the functional properties of the resulting protein molecule. It is therefore understood that the invention encompasses more than the specific exemplary nucleotide or amino acid sequences and includes functional equivalents thereof. The terms "substantially similar" and "corresponding substantially" are used interchangeably herein.

Substantially similar nucleic acid fragments may be selected by screening nucleic acid fragments representing subfragments or modifications of the nucleic acid fragments of the instant invention, wherein one or more nucleotides are substituted, deleted and/or inserted, for their ability to affect the level of the polypeptide encoded by the unmodified nucleic acid fragment in a plant or plant cell. For example, a substantially similar nucleic acid fragment representing at least one of 30 contiguous nucleotides derived from the instant nucleic acid fragment can be constructed and introduced into a plant or plant cell. The level of the polypeptide encoded by the unmodified nucleic acid fragment present in a plant or plant cell exposed to the substantially similar nucleic fragment can then be compared to the level of the polypeptide in a plant or plant cell that is not exposed to the substantially similar nucleic acid fragment.

For example, it is well known in the art that antisense suppression and co-suppression of gene expression may be accomplished using nucleic acid fragments representing less than the entire coding region of a gene, and by using nucleic acid fragments that do not share 100% sequence identity with the gene to be suppressed. Moreover, alterations in a nucleic acid fragment which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded polypeptide, are well known in the art. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products. Consequently, an isolated polynucleotide comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, and 35, and the complement of such nucleotide sequences may be used in methods of selecting an isolated polynucleotide that affects the expression of a COI1 or an LLS1 protein in a host cell. A method of selecting an isolated polynucleotide that affects the level of expression of a polypeptide in a virus or in a host cell (eukaryotic, such as plant or yeast, prokaryotic such as bacterial) may comprise the steps of: constructing an isolated polynucleotide of the present invention or a chimeric gene of the present invention; introducing the isolated polynucleotide or the chimeric gene into a host cell; measuring the level of a polypeptide or enzyme activity in the host cell containing the isolated

polynucleotide; and comparing the level of a polypeptide or enzyme activity in the host cell containing the isolated polynucleotide with the level of a polypeptide or enzyme activity in a host cell that does not contain the isolated polynucleotide.

Moreover, substantially similar nucleic acid fragments may also be characterized by their ability to hybridize. Estimates of such homology are provided by either DNA-DNA or DNA-RNA hybridization under conditions of stringency as is well understood by those skilled in the art (Hames and Higgins, Eds. (1985) Nucleic Acid Hybridisation, IRL Press, Oxford, U.K.). Stringency conditions can be adjusted to screen for moderately similar fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. Post-hybridization washes determine stringency conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 min, then repeated with 2X SSC, 0.5% SDS at 45°C for 30 min, and then repeated twice with 0.2X SSC, 0.5% SDS at 50°C for 30 min. A more preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5% SDS was increased to 60°C. Another preferred set of highly stringent conditions uses two final washes in 0.1X SSC, 0.1% SDS at 65°C.

Substantially similar nucleic acid fragments of the instant invention may also be characterized by the percent identity of the amino acid sequences that they encode to the amino acid sequences disclosed herein, as determined by algorithms commonly employed by those skilled in this art. Suitable nucleic acid fragments (isolated polynucleotides of the present invention) encode polypeptides that are at least about 70% identical, preferably at least about 80% identical to the amino acid sequences reported herein. Preferred nucleic acid fragments encode amino acid sequences that are about 85% identical to the amino acid sequences reported herein. More preferred nucleic acid fragments encode amino acid sequences that are at least about 90% identical to the amino acid sequences reported herein. Most preferred are nucleic acid fragments that encode amino acid sequences that are at least about 95% identical to the amino acid sequences reported herein. Suitable nucleic acid fragments not only have the above identities but typically encode a polypeptide having at least 50 amino acids, preferably at least 100 amino acids, more preferably at least 150 amino acids, still more preferably at least 200 amino acids, and most preferably at least 250 amino acids. Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS.* 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for

pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

A "substantial portion" of an amino acid or nucleotide sequence comprises an amino acid or a nucleotide sequence that is sufficient to afford putative identification of the protein or gene that the amino acid or nucleotide sequence comprises. Amino acid and nucleotide sequences can be evaluated either manually by one skilled in the art, or by using computer-based sequence comparison and identification tools that employ algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol. 215*:403-410; see also www.ncbi.nlm.nih.gov/BLAST/). In general, a sequence of ten or more contiguous amino acids or thirty or more contiguous nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene-specific oligonucleotide probes comprising 30 or more contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12 or more nucleotides may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises a nucleotide sequence that will afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches amino acid and nucleotide sequences encoding polypeptides that comprise one or more particular plant proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

"Codon degeneracy" refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment comprising a nucleotide sequence that encodes all or a substantial portion of the amino acid sequences set forth herein. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a nucleic acid fragment for improved expression in a host cell, it is desirable to design the nucleic acid fragment such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

"Synthetic nucleic acid fragments" can be assembled from oligonucleotide building blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form larger nucleic acid fragments which may then be enzymatically assembled to construct the entire desired nucleic acid fragment.

"Chemically synthesized", as related to a nucleic acid fragment, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of nucleic acid fragments may be accomplished using well established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the nucleic acid fragments can be tailored for optimal gene expression based on optimization of the nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

"Gene" refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. "Endogenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign gene" refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

"Coding sequence" refers to a nucleotide sequence that codes for a specific amino acid sequence. "Regulatory sequences" refers to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

"Promoter" refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an "enhancer" is a nucleotide sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or may be composed of different elements derived from different promoters found in nature, or may even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or

at different stages of development, or in response to different environmental conditions. Promoters which cause a nucleic acid fragment to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants 15*:1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleic acid fragments of different lengths may have identical promoter activity.

"Translation leader sequence" refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Mol. Biotechnol. 3*:225-236).

"3' Non-coding sequences" refers to nucleotide sequences located downstream of a coding sequence and includes polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al. (1989) *Plant Cell 1*:671-680.

"RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. "Messenger RNA (mRNA)" refers to the RNA that is without introns and can be translated into polypeptides by the cell. "cDNA" refers to DNA that is complementary to and derived from an mRNA template. The cDNA can be single-stranded or converted to double stranded form using, for example, the Klenow fragment of DNA polymerase I. "Sense RNA" refers to an RNA transcript that includes the mRNA and can be translated into a polypeptide by the cell. "Antisense RNA" refers to an RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (see U.S. Patent No. 5,107,065, incorporated herein by reference). The complementarity of an antisense RNA may be with any part of the specific nucleotide sequence, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. "Functional RNA" refers to sense RNA, antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes.

The term "operably linked" refers to the association of two or more nucleic acid fragments so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. "Expression" may also refer to the translation of mRNA into a polypeptide. "Antisense inhibition" refers to the production of antisense RNA transcripts capable of suppressing the expression of the target protein. "Overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms. "Co-suppression" refers to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar foreign or endogenous genes (U.S. Patent No. 5,231,020, incorporated herein by reference).

A "protein" or "polypeptide" is a chain of amino acids arranged in a specific order determined by the coding sequence in a polynucleotide encoding the polypeptide. Each protein or polypeptide has a unique function.

"Altered levels" or "altered expression" refer to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

"Null mutant" refers to a host cell which either lacks the expression of a certain polypeptide or expresses a polypeptide which is inactive or does not have any detectable expected enzymatic function.

"Mature protein" or the term "mature" when used in describing a protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed. "Precursor protein" or the term "precursor" when used in describing a protein refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

A "chloroplast transit peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the chloroplast or other plastid types present in the cell in which the protein is made. "Chloroplast transit sequence" refers to a nucleotide sequence that encodes a chloroplast transit peptide. A "signal peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the secretory system (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol. 42*:21-53). If the protein is to be directed to a vacuole, a vacuolar targeting signal (*supra*) can further be added, or if to the endoplasmic reticulum, an endoplasmic reticulum retention signal (*supra*)

may be added. If the protein is to be directed to the nucleus, any signal peptide present should be removed and instead a nuclear localization signal included (Raikhel (1992) *Plant Phys. 100*:1627-1632).

"Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol. 143*:277) and particle-accelerated or "gene gun" transformation technology (Klein et al. (1987) *Nature (London) 327*:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference). Thus, isolated polynucleotides of the present invention can be incorporated into recombinant constructs, typically DNA constructs, capable of introduction into and replication in a host cell. Such a construct can be a vector that includes a replication system and sequences that are capable of transcription and translation of a polypeptide-encoding sequence in a given host cell. A number of vectors suitable for stable transfection of plant cells or for the establishment of transgenic plants have been described in, e.g., Pouwels et al., Cloning Vectors: A Laboratory Manual, 1985, supp. 1987; Weissbach and Weissbach, Methods for Plant Molecular Biology, Academic Press, 1989; and Flevin et al., Plant Molecular Biology Manual, Kluwer Academic Publishers, 1990. Typically, plant expression vectors include, for example, one or more cloned plant genes under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant expression vectors also can contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook et al. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter "Maniatis").

"PCR" or "polymerase chain reaction" is well known by those skilled in the art as a technique used for the amplification of specific DNA segments (U.S. Patent Nos. 4,683,195 and 4,800,159).

The present invention concerns an isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of: (a) a first nucleotide sequence encoding a polypeptide of at least 60 amino acids selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36, or (b) a second nucleotide sequence comprising the complement of the first nucleotide sequence.

Preferably, the first nucleotide sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, and 35.

Nucleic acid fragments encoding at least a substantial portion of several disease resistance factors have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding homologous proteins from the same or other plant species. Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction, ligase chain reaction).

For example, genes encoding other COI1 or LLS1 proteins, either as cDNAs or genomic DNAs, could be isolated directly by using all or a substantial portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, an entire sequence(s) can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA 85*:8998-9002) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems

(BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA 86*:5673-5677; Loh et al. (1989) *Science 243*:217-220). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques 1*:165). Consequently, a polynucleotide comprising a nucleotide sequence of at least one of 60 (preferably one of at least 40, most preferably one of at least 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, and 35 and the complement of such nucleotide sequences may be used in such methods to obtain a nucleic acid fragment encoding a substantial portion of an amino acid sequence of a polypeptide.

The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a COI1 or an LLS1 protein, preferably a substantial portion of a plant COI1 or LLS1 protein, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, and 35, and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a portion of a COI1 or an LLS1 protein.

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing substantial portions of the instant amino acid sequences may be synthesized. These peptides can be used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins comprising the amino acid sequences. These antibodies can be then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner (1984) *Adv. Immunol. 36*:1-34; Maniatis).

In another embodiment, this invention concerns viruses and host cells comprising either the chimeric genes of the invention as described herein or an isolated polynucleotide of the invention as described herein. Examples of host cells which can be used to practice the invention include, but are not limited to, yeast, bacteria, and plants.

As was noted above, the nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed polypeptides are present at higher or lower levels than normal or in cell types or developmental stages in which they are not normally found. This would have the effect of altering the level of disease resistance in those cells. LLS1 is a suppressor of cell death, thus decreasing its production will result in cell death. Overexpression of COI1 should induce systemic resistance to a broad range of pathogens.

Overexpression of the proteins of the instant invention may be accomplished by first constructing a chimeric gene in which the coding region is operably linked to a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. The chimeric gene may comprise promoter sequences and translation leader sequences derived from the same genes. 3' Non-coding sequences encoding transcription termination signals may also be provided. The instant chimeric gene may also comprise one or more introns in order to facilitate gene expression.

Plasmid vectors comprising the instant isolated polynucleotide (or chimeric gene) may be constructed. The choice of plasmid vector is dependent upon the method that will be used to transform host plants. The skilled artisan is well aware of the genetic elements that must be present on the plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al. (1985) *EMBO J. 4*:2411-2418; De Almeida et al. (1989) *Mol. Gen. Genetics 218*:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis.

For some applications it may be useful to direct the instant polypeptides to different cellular compartments, or to facilitate secretion from the cell. It is thus envisioned that the chimeric gene described above may be further supplemented by directing the coding sequence to encode the instant polypeptides with appropriate intracellular targeting sequences such as transit sequences (Keegstra (1989) *Cell 56*:247-253), signal sequences or sequences encoding endoplasmic reticulum localization (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol. 42*:21-53), or nuclear localization signals (Raikhel (1992) *Plant Phys.100*:1627-1632) with or without removing targeting sequences that are already present. While the references cited give examples of each of these, the list is not exhaustive and more targeting signals of use may be discovered in the future.

In another embodiment, the present invention concerns a polypeptide of at least 60 amino acids selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36.

The instant polypeptides (or substantial portions thereof) may be produced in heterologous host cells, particularly in the cells of microbial hosts, and can be used to prepare antibodies to these proteins by methods well known to those skilled in the art. The antibodies are useful for detecting the polypeptides of the instant invention *in situ* in cells or *in vitro* in cell extracts. Preferred heterologous host cells for production of the instant polypeptides are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well

known to those skilled in the art. Any of these could be used to construct a chimeric gene for production of the instant polypeptides. This chimeric gene could then be introduced into appropriate microorganisms via transformation to provide high level expression of the encoded disease resistance factor. An example of a vector for high level expression of the instant polypeptides in a bacterial host is provided (Example 7).

Additionally, the instant LLS1 protein can be used as a target to facilitate design and/or identification of inhibitors of those enzymes that may be useful as herbicides. This is desirable because the LLS1 described herein catalyzes inactivation of the cell-death signal. Accordingly, inhibition of the activity of LLS1 could lead to inhibition plant growth. Thus, the instant LLS1 could be appropriate for new herbicide discovery and design.

All or a substantial portion of the polynucleotides of the instant invention may also be used as probes for genetically and physically mapping the genes that they are a part of, and used as markers for traits linked to those genes. Such information may be useful in plant breeding in order to develop lines with desired phenotypes. For example, the instant nucleic acid fragments may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots (Maniatis) of restriction-digested plant genomic DNA may be probed with the nucleic acid fragments of the instant invention. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al. (1987) *Genomics 1*:174-181) in order to construct a genetic map. In addition, the nucleic acid fragments of the instant invention may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of individuals representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is noted and used to calculate the position of the instant nucleic acid sequence in the genetic map previously obtained using this population (Botstein et al. (1980) *Am. J. Hum. Genet. 32*:314-331).

The production and use of plant gene-derived probes for use in genetic mapping is described in Bernatzky and Tanksley (1986) *Plant Mol. Biol. Reporter 4*:37-41. Numerous publications describe genetic mapping of specific cDNA clones using the methodology outlined above or variations thereof. For example, F2 intercross populations, backcross populations, randomly mated populations, near isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

Nucleic acid probes derived from the instant nucleic acid sequences may also be used for physical mapping (i.e., placement of sequences on physical maps; *see* Hoheisel et al. In: *Nonmammalian Genomic Analysis: A Practical Guide*, Academic press 1996, pp. 319-346, and references cited therein).

In another embodiment, nucleic acid probes derived from the instant nucleic acid sequences may be used in direct fluorescence *in situ* hybridization (FISH) mapping (Trask (1991) *Trends Genet. 7*:149-154). Although current methods of FISH mapping favor use of

large clones (several to several hundred KB; see Laan et al. (1995) *Genome Res.* 5:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

A variety of nucleic acid amplification-based methods of genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include allele-specific amplification (Kazazian (1989) *J. Lab. Clin. Med. 11*:95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield et al. (1993) *Genomics 16*:325-332), allele-specific ligation (Landegren et al. (1988) *Science 241*:1077-1080), nucleotide extension reactions (Sokolov (1990) *Nucleic Acid Res. 18*:3671), Radiation Hybrid Mapping (Walter et al. (1997) *Nat. Genet. 7*:22-28) and Happy Mapping (Dear and Cook (1989) *Nucleic Acid Res. 17*:6795-6807). For these methods, the sequence of a nucleic acid fragment is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

Loss of function mutant phenotypes may be identified for the instant cDNA clones either by targeted gene disruption protocols or by identifying specific mutants for these genes contained in a maize population carrying mutations in all possible genes (Ballinger and Benzer (1989) *Proc. Natl. Acad. Sci USA 86*:9402-9406; Koes et al. (1995) *Proc. Natl. Acad. Sci USA 92*:8149-8153; Bensen et al. (1995) *Plant Cell 7*:75-84). The latter approach may be accomplished in two ways. First, short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols in conjunction with a mutation tag sequence primer on DNAs prepared from a population of plants in which Mutator transposons or some other mutation-causing DNA element has been introduced (see Bensen, *supra*). The amplification of a specific DNA fragment with these primers indicates the insertion of the mutation tag element in or near the plant gene encoding the instant polypeptides. Alternatively, the instant nucleic acid fragment may be used as a hybridization probe against PCR amplification products generated from the mutation population using the mutation tag sequence primer in conjunction with an arbitrary genomic site primer, such as that for a restriction enzyme site-anchored synthetic adaptor. With either method, a plant containing a mutation in the endogenous gene encoding the instant polypeptides can be identified and obtained. This mutant plant can then be used to determine or confirm the natural function of the instant polypeptides disclosed herein.

EXAMPLES

The present invention is further defined in the following Examples, in which parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention,

are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

The disclosure of each reference set forth herein is incorporated herein by reference in its entirety.

EXAMPLE 1

Composition of cDNA Libraries; Isolation and Sequencing of cDNA Clones

cDNA libraries representing mRNAs from various corn, rice, soybean, and wheat tissues were prepared. The characteristics of the libraries are described below.

TABLE 2

cDNA Libraries from Corn, Rice, Soybean, and Wheat

Library	Tissue	Clone
p0037	Corn V5 Stage* Roots Infested With Corn Root Worm	p0037.crwbs69r
p0128	Corn Primary and Secondary Immature Ear, Pooled	p0128.cpici34r
rds2c	Rice Developing Seeds From Middle of the Plant	rds2c.pk005.b12
rl0n	Rice 15 Day Old Leaf**	rl0n.pk0047.c5
rl0n	Rice 15 Day Old Leaf**	rl0n.pk099.p14
rlr2	Rice Leaf 15 Days After Germination, 2 Hours After Infection of Strain *Magaporthe grisea* 4360-R-62 (AVR2-YAMO); Resistant	rlr2.pk0027.h4
sgc2c	Soybean Cotyledon 12-20 Days After Germination (Mature Green)	sgc2c.pk001.c22
sgs4c	Soybean Seeds 2 Days After Germination	sgs4c.pk003.k23
wl1n	Wheat Leaf From 7 Day Old Seedling**	wl1n.pk0018.f8
wl1n	Wheat Leaf From 7 Day Old Seedling**	wl1n.pk0049.f7
wlm0	Wheat Seedlings 0 Hour After Inoculation With *Erysiphe graminis f. sp tritici*	wlm0.pk0002.c10
wlm0	Wheat Seedlings 0 Hour After Inoculation With *Erysiphe graminis f. sp tritici*	wlm0.pk0009.d7
wlmk1	Wheat Seedlings 1 Hour After Inoculation With *Erysiphe graminis f. sp tritici* and Treatment With Herbicide***	wlmk1.pk0015.h3
wre1n	Wheat Root From 7 Day Old Etiolated Seedling**	wre1n.pk0122.d3

* Corn developmental stages are explained in the publication "How a corn plant develops" from the Iowa State University Coop. Ext. Service Special Report No. 48 reprinted June 1993.

** These libraries were normalized essentially as described in U.S. Patent No. 5,482,845, incorporated herein by reference.

***Application of 6-iodo-2-propoxy-3-propyl-4(3*H*)-quinazolinone; synthesis and methods of using this compound are described in U.S. Patent No. 5,747,497, incorporated herein by reference.

cDNA libraries may be prepared by any one of many methods available. For example, the cDNAs may be introduced into plasmid vectors by first preparing the cDNA libraries in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA). The Uni-ZAP™ XR libraries are converted into plasmid libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts will be contained in the plasmid vector pBluescript. In addition, the cDNAs may be introduced directly into precut Bluescript II SK(+) vectors (Stratagene) using T4 DNA ligase (New England Biolabs), followed by transfection into DH10B cells according to the manufacturer's protocol (GIBCO BRL Products). Once the cDNA inserts are in plasmid vectors, plasmid DNAs are prepared from randomly picked bacterial colonies containing recombinant pBluescript plasmids, or the insert cDNA sequences are amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Amplified insert DNAs or plasmid DNAs are sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams et al., (1991) *Science 252*:1651-1656). The resulting ESTs are analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

EXAMPLE 2

Identification of cDNA Clones

cDNA clones encoding disease resistance factors were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol. 215*:403-410; see also www.ncbi.nlm.nih.gov/BLAST/) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish and States (1993) *Nat. Genet. 3*:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the

reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

EXAMPLE 3

Characterization of cDNA Clones Encoding COI1

The BLASTX search using the EST sequences from clones listed in Table 3 revealed similarity of the polypeptides encoded by the Contig to an unknown protein from chromosome 2 of *Arabidopsis thaliana* (NCBI General Identifier No. 2088647) which is identical to the polypeptides encoded by the cDNA to COI1 protein from *Arabidopsis thaliana* (NCBI General Identifier No. 3158394). Shown in Table 3 are the BLAST results for individual ESTs ("EST"), or for the sequences of contigs assembled from two or more ESTs ("Contig"):

TABLE 3

BLAST Results for Clones Encoding Polypeptides Homologous to COI1

Clone	Status	BLAST pLog Score 2088647 or 3158394
p0128.cpici34r	EST	42.30
Contig of: rlr2.pk0027.h4 rl0n.pk099.p14 rl0n.pk0047.c5	Contig	104.00
sgs4c.pk003.k23	EST	48.00
Contig of: wre1n.pk0122.d3 wl1n.pk0018.f8	Contig	76.00

Further sequencing allowed the determination of the sequence of the entire cDNA insert in clones p0128.cpici34r, rl0n.pk099.p14, sgs4c.pk003.k23, and wre1n.pk0122.d3. Further searching of the DuPont proprietary database allowed the identification of other corn and wheat ESTs with similarities to COI1. The BLASTX search using the EST sequences or the BLASTP search using the amino acid sequences encoded by the entire cDNA inserts from clones listed in Table 3 revealed similarity of the polypeptides encoded by the Contig to an unknown protein from chromosome 2 of *Arabidopsis thaliana* (NCBI General Identifier No. 2088647) which is identical to the polypeptides encoded by the cDNA to COI1 protein from *Arabidopsis thaliana* (NCBI General Identifier No. 3158394). Shown in Table 4 are the BLAST results for individual ESTs ("EST"), for the amino acid sequences derived from the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), or from the amino acid sequences of the entire polypeptide derived from an FIS or an FIS and PCR ("CGS"):

TABLE 4

BLAST Results for Sequences Encoding Polypeptides Homologous to COI1

Clone	Status	BLAST pLog Score 2088647 or 3158394
p0037.crwbs69r	EST	38.00
p0128.cpici34r:fis	FIS	135.00
rl0n.pk099.p14:fis	CGS	>254.00
sgs4c.pk003.k23:fis	CGS	>254.00
wl1n.pk0049.f7	EST	7.00
wlm0.pk0009.d7	EST	15.40
wre1n.pk0122.d3:fis	FIS	75.70

Figure 1 presents an alignment of the amino acid sequences set forth in SEQ ID NOs:18, 20, and 22 and the *Arabidopsis thaliana* sequence (NCBI General Identifier No. 3158394; SEQ ID NO:37). Underlined amino acids in SEQ ID NO:37 correspond to the degenerate F-box motif and the 16 imperfect leucine-rich repeats (LRRs) indicated by Xie et al. (1998, *Science 280*:1091-1094). The data in Table 5 presents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6, 8, 16, 18, 20, 22, 24, 26, and 28 and the *Arabidopsis thaliana* COI1 protein sequence (SEQ ID NO:37).

TABLE 5

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to COI1

SEQ ID NO.	Percent Identity to 2088647 (SEQ ID NO:37)
2	42.3
4	68.2
6	74.1
8	67.2
16	39.2
18	52.0
20	55.4
22	67.7
24	28.9
26	29.9
28	69.4

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc.,

Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS.* 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments, BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode substantial portions of a corn, a rice, a soybean, and a wheat COI1 protein and the entire rice and soybean COI1 proteins.

EXAMPLE 4

Characterization of cDNA Clones Encoding LLS1

The BLASTX search using the EST sequences from clones listed in Table 6 revealed similarity of the polypeptides encoded by the cDNAs to LLS1 from *Zea mays* (NCBI General Identifier No. 1935912) and by the contig to LLS1 from *Arabidopsis thaliana* (NCBI General Identifier No. 1935914). Shown in Table 6 are the organisms from which the closest art sequence is derived from, the NCBI General Identifier Number, and BLAST results for individual ESTs ("EST"):

TABLE 6

BLAST Results for Clones Encoding Polypeptides Homologous to LLS1

Clone	Status	Organism	NCBI gi No.	BLAST pLog Score
rds2c.pk005.b12	EST	*Zea mays*	1935912	53.30
sgc2c.pk001.c22	EST	*Arabidopsis thaliana*	1935914	28.30
wlmk1.pk0015.h3	EST	*Zea mays*	1935912	68.70

The sequence of the entire cDNA insert from the clones listed in Table 6 was determined. Further searching of the DuPont proprietary database allowed the identification of another, more 5', LLS1-encoding wheat clone. The BLASTX search using the EST sequences or the BLASTP search using the amino acid sequences encoded by the entire cDNA inserts from clones listed in Table 6 revealed similarity of the polypeptides encoded by the cDNAs to LLS1 from *Zea mays* (NCBI General Identifier Nos. 1935912 and 7489721) and by the contig to LLS1 from *Arabidopsis thaliana* (NCBI General Identifier No. 1935914). The two *Zea mays* amino acid sequences are identical through 505 amino acids. The amino acid sequence having NCBI General Identifier No. 7489721 contains 15 extra amino acids at the C-terminus compared to the amino acid sequence presented in NCBI General Identifier No. 1935912. Shown in Table 7 are the organisms from which the closest art sequence is derived from, the NCBI General Identifier Number, and the BLAST results for individual ESTs ("EST"), for the amino acid sequences derived from the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), or for

the amino acid sequences of the entire polypeptide derived from an FIS or an FIS and PCR ("CGS"):

TABLE 7

BLAST Results for Sequences Encoding Polypeptides Homologous to LLS1

Clone	Status	Organism	NCBI gi No.	BLAST pLog Score
rds2c.pk005.b12:fis	FIS	*Zea mays*	1935912	>254.00
sgc2c.pk001.c22:fis	CGS	*Arabidopsis thaliana*	1935914	>254.00
wlm0.pk0002.c10	EST	*Zea mays*	1935912	7.00
wlmk1.pk0015.h3:fis	FIS	*Zea mays*	7489721	>254.00

Figure 2 presents an alignment of the amino acid sequences set forth in SEQ ID NOs:30, 32, and 36 and the *Zea mays* sequence (NCBI General Identifier Nos. 7489721, SEQ ID NO:38). Underlined amino acids in SEQ ID NO:38 correspond to the consensus sequence for coordinating the Reiske-type [2Fe-2S] cluster and the mononuclear non-heme binding site (Gray et al. (1997) *Cell 89*:25-31) The data in Table 8 presents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:10, 12, 14, 30, 32, and 36 and the *Zea mays* sequence (SEQ ID NO:38).

TABLE 8

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to LLS1

SEQ ID NO.	Percent Identity to 7489721 (SEQ ID NO:38)
10	79.4
12	65.2
14	86.7
30	83.1
32	67.9
34	22.9
36	85.8

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS. 5*:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments, BLAST scores and

probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode a substantial portion and an entire soybean an rice LLS1 proteins and two portions and a substantial portion wheat LLS1 protein.

EXAMPLE 5

Expression of Chimeric Genes in Monocot Cells

A chimeric gene comprising a cDNA encoding the instant polypeptides in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on an agarose gel. The appropriate band can be isolated from the gel and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb SalI-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-SalI fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli* XL1-Blue (Epicurian Coli XL-1 Blue™; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase™ DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding the instant polypeptides, and the 10 kD zein 3' region.

The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al. (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

The particle bombardment method (Klein et al. (1987) *Nature* 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles (1 μm in diameter) are coated with DNA using the following technique. Ten μg of plasmid DNAs are added to 50 μL of a suspension of gold particles (60 mg per mL). Calcium chloride (50 μL of a 2.5 M solution) and spermidine free base (20 μL of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 μL of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30 μL of ethanol. An aliquot (5 μL) of the DNA-coated gold particles can be placed in the center of a Kapton™ flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a Biolistic™ PDS-1000/He (Bio-Rad Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of mercury. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

Seven days after bombardment the tissue can be transferred to N6 medium that contains gluphosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing gluphosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the

tissue can be transferred to regeneration medium (Fromm et al. (1990) *Bio/Technology 8*:833-839).

EXAMPLE 6

Expression of Chimeric Genes in Dicot Cells

A seed-specific construct composed of the promoter and transcription terminator from the gene encoding the β subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem. 261*:9228-9238) can be used for expression of the instant polypeptides in transformed soybean. The phaseolin construct includes about 500 nucleotides upstream (5') from the translation initiation codon and about 1650 nucleotides downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I (which includes the ATG translation initiation codon), Sma I, Kpn I and Xba I. The entire construct is flanked by Hind III sites.

The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the expression vector. Amplification is then performed as described above, and the isolated fragment is inserted into a pUC18 vector carrying the seed construct.

Soybean embryos may then be transformed with the expression vector comprising sequences encoding the instant polypeptides. To induce somatic embryos, cotyledons, 3-5 mm in length dissected from surface sterilized, immature seeds of the soybean cultivar A2872, can be cultured in the light or dark at 26°C on an appropriate agar medium for 6-10 weeks. Somatic embryos which produce secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos which multiplied as early, globular staged embryos, the suspensions are maintained as described below.

Soybean embryogenic suspension cultures can be maintained in 35 mL liquid media on a rotary shaker, 150 rpm, at 26°C with florescent lights on a 16:8 hour day/night schedule. Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein et al. (1987) *Nature* (London) *327*:70-73, U.S. Patent No. 4,945,050). A DuPont Biolistic™ PDS1000/HE instrument (helium retrofit) can be used for these transformations.

A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature 313*:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz et al.(1983) *Gene 25*:179-188) and the 3' region of the nopaline synthase

gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The seed construct comprising the phaseolin 5' region, the fragment encoding the instant polypeptides and the phaseolin 3' region can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

To 50 μL of a 60 mg/mL 1 μm gold particle suspension is added (in order): 5 μL DNA (1 μg/μL), 20 μL spermidine (0.1 M), and 50 μL $CaCl_2$ (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the supernatant removed. The DNA-coated particles are then washed once in 400 μL 70% ethanol and resuspended in 40 μL of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five μL of the DNA-coated gold particles are then loaded on each macro carrier disk.

Approximately 300-400 mg of a two-week-old suspension culture is placed in an empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette. For each transformation experiment, approximately 5-10 plates of tissue are normally bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches of mercury. The tissue is placed approximately 3.5 inches away from the retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.

EXAMPLE 7

Expression of Chimeric Genes in Microbial Cells

The cDNAs encoding the instant polypeptides can be inserted into the T7 *E. coli* expression vector pBT430. This vector is a derivative of pET-3a (Rosenberg et al. (1987) *Gene 56*:125-135) which employs the bacteriophage T7 RNA polymerase/T7 promoter system. Plasmid pBT430 was constructed by first destroying the EcoR I and Hind III sites in pET-3a at their original positions. An oligonucleotide adaptor containing EcoR I and Hind III sites was inserted at the BamH I site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then, the Nde I site at the position of translation initiation was converted to an Nco I site using

oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the protein. This fragment may then be purified on a 1% NuSieve GTG™ low melting agarose gel (FMC). Buffer and agarose contain 10 μg/mL ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the agarose gel by digestion with GELase™ (Epicentre Technologies) according to the manufacturer's instructions, ethanol precipitated, dried and resuspended in 20 μL of water. Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase (New England Biolabs, Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector pBT430 is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized with phenol/chloroform as described above. The prepared vector pBT430 and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent cells (GIBCO BRL). Transformants can be selected on agar plates containing LB media and 100 μg/mL ampicillin. Transformants containing the gene encoding the instant polypeptides are then screened for the correct orientation with respect to the T7 promoter by restriction enzyme analysis.

For high level expression, a plasmid clone with the cDNA insert in the correct orientation relative to the T7 promoter can be transformed into *E. coli* strain BL21(DE3) (Studier et al. (1986) *J. Mol. Biol. 189*:113-130). Cultures are grown in LB medium containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio-β-galactoside, the inducer) can be added to a final concentration of 0.4 mM and incubation can be continued for 3 h at 25°. Cells are then harvested by centrifugation and re-suspended in 50 μL of 50 mM Tris-HCl at pH 8.0 containing 0.1 mM DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant determined. One μg of protein from the soluble fraction of the culture can be separated by SDS-polyacrylamide gel electrophoresis. Gels can be observed for protein bands migrating at the expected molecular weight.

EXAMPLE 8

Evaluating Compounds for Their Ability to Inhibit the Activity of LLS1

The LLS1 polypeptide described herein may be produced using any number of methods known to those skilled in the art. Such methods include, but are not limited to, expression in bacteria as described in Example 7, or expression in eukaryotic cell culture, *in planta*, and using viral expression systems in suitably infected organisms or cell lines. The instant LLS1 polypeptide may be expressed either as mature forms of the proteins as

observed *in vivo* or as fusion proteins by covalent attachment to a variety of enzymes, proteins or affinity tags. Common fusion protein partners include glutathione S-transferase ("GST"), thioredoxin ("Trx"), maltose binding protein, and C- and/or N-terminal hexahistidine polypeptide ("$(His)_6$"). The fusion proteins may be engineered with a protease recognition site at the fusion point so that fusion partners can be separated by protease digestion to yield intact mature enzyme. Examples of such proteases include thrombin, enterokinase and factor Xa. However, any protease can be used which specifically cleaves the peptide connecting the fusion protein and the enzyme.

Purification of the instant LLS1 polypeptide, if desired, may utilize any number of separation technologies familiar to those skilled in the art of protein purification. Examples of such methods include, but are not limited to, homogenization, filtration, centrifugation, heat denaturation, ammonium sulfate precipitation, desalting, pH precipitation, ion exchange chromatography, hydrophobic interaction chromatography and affinity chromatography, wherein the affinity ligand represents a substrate, substrate analog or inhibitor. When the instant LLS1 polypeptide are expressed as fusion proteins, the purification protocol may include the use of an affinity resin which is specific for the fusion protein tag attached to the expressed enzyme or an affinity resin containing ligands which are specific for the enzyme. For example, the instant LLS1 polypeptide may be expressed as a fusion protein coupled to the C-terminus of thioredoxin. In addition, a $(His)_6$ peptide may be engineered into the N-terminus of the fused thioredoxin moiety to afford additional opportunities for affinity purification. Other suitable affinity resins could be synthesized by linking the appropriate ligands to any suitable resin such as Sepharose-4B. In an alternate embodiment, a thioredoxin fusion protein may be eluted using dithiothreitol; however, elution may be accomplished using other reagents which interact to displace the thioredoxin from the resin. These reagents include β–mercaptoethanol or other reduced thiol. The eluted fusion protein may be subjected to further purification by traditional means as stated above, if desired. Proteolytic cleavage of the thioredoxin fusion protein and the enzyme may be accomplished after the fusion protein is purified or while the protein is still bound to the ThioBond™ affinity resin or other resin.

Crude, partially purified or purified enzyme, either alone or as a fusion protein, may be utilized in assays for the evaluation of compounds for their ability to inhibit enzymatic activation of the instant LLS1 polypeptide disclosed herein. Assays may be conducted under well known experimental conditions which permit optimal enzymatic activity. For example, assays for LLS1 are presented by Gray et al. (1997) *Cell 89*:25-31.

CLAIMS

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:

(a) a first nucleotide sequence encoding a polypeptide of at least 60 amino acids selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36; or

(b) a second nucleotide sequence comprising a complement of the first nucleotide sequence.

2. The isolated polynucleotide of Claim 1, wherein the first nucleotide sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, and 35.

3. The isolated polynucleotide of Claim 1 wherein the nucleotide sequences are DNA.

4. The isolated polynucleotide of Claim 1 wherein the nucleotide sequences are RNA.

5. A chimeric gene comprising the isolated polynucleotide of Claim 1 operably linked to at least one suitable regulatory sequence.

6. A host cell comprising the chimeric gene of Claim 5.

7. A host cell comprising the isolated polynucleotide of Claim 1.

8. The host cell of Claim 7 wherein the host cell is selected from the group consisting of yeast, bacteria, and plant.

9. A virus comprising the isolated polynucleotide of Claim 1.

10. A polypeptide of at least 60 amino acids selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36.

11. A method of selecting an isolated polynucleotide that affects the level of expression of a disease resistance factor polypeptide in a plant cell, the method comprising the steps of:

(a) constructing the isolated polynucleotide comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from the isolated polynucleotide of Claim 1;

(b) introducing the isolated polynucleotide into the plant cell;

(c) measuring the level of the polypeptide in the plant cell containing the polynucleotide; and

(d) comparing the level of the polypeptide in the plant cell containing the isolated polynucleotide with the level of the polypeptide in a plant cell that does not contain the isolated polynucleotide.

12. The method of Claim 11 wherein the isolated polynucleotide comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, and 35.

13. A method of selecting an isolated polynucleotide that affects the level of expression of a disease resistance factor polypeptide in a plant cell, the method comprising the steps of:

(a) constructing the isolated polynucleotide of Claim 1;

(b) introducing the isolated polynucleotide into the plant cell;

(c) measuring the level of the polypeptide in the plant cell containing the polynucleotide; and

(d) comparing the level of the polypeptide in the plant cell containing the isolated polynucleotide with the level of the polypeptide in a plant cell that does not contain the polynucleotide.

14. A method of obtaining a nucleic acid fragment encoding a disease resistance factor polypeptide comprising the steps of:

(a) synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, and 35 and a complement of such nucleotide sequences; and

(b) amplifying a nucleic acid sequence using the oligonucleotide primer.

15. A method of obtaining a nucleic acid fragment encoding a disease resistance factor polypeptide comprising the steps of:

(a) probing a cDNA or genomic library with an isolated polynucleotide comprising at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, and 35 and a complement of such nucleotide sequences;

(b) identifying a DNA clone that hybridizes with the isolated polynucleotide;

(c) isolating the identified DNA clone; and

(d) sequencing a cDNA or genomic fragment that comprises the isolated DNA clone.

16. A composition comprising the isolated polynucleotide of Claim 1.

17. A composition comprising the isolated polypeptide of Claim 10.

18. The isolated polynucleotide of Claim 1 comprising a nucleotide sequence having at least one of 30 contiguous nucleotides.

19. A method for positive selection of a transformed cell comprising:

(a) transforming a host cell with the chimeric gene of Claim 5; and

(b) growing the transformed host cell under conditions which allow expression of a polynucleotide in an amount sufficient to complement a null mutant to provide a positive selection means.

20. The method of Claim 19 wherein the host cell is a plant.

21. The method of Claim 20 wherein the plant cell is a monocot.

22. The method of Claim 20 wherein the plant cell is a dicot.

23. A method of altering the level of expression of a disease resistance factor in a host cell comprising:

(a) transforming the host cell with the chimeric gene of Claim 5; and

(b) growing the transformed host cell produced in step (a) under conditions that are suitable for expression of the chimeric gene

wherein expression of the chimeric gene results in production of altered levels of the disease resistance factor in the transformed host cell.

24. A method for evaluating at least one compound for its ability to inhibit the activity of an LLS1 protein, the method comprising the steps of:

(a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding the LLS1 protein, operably linked to at least one suitable regulatory sequence;

(b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the LLS1 protein encoded by the operably linked nucleic acid fragment in the transformed host cell;

(c) optionally purifying the LLS1 protein expressed by the transformed host cell;

(d) treating the LLS1 protein polypeptide with a compound to be tested; and

(e) comparing the activity of the LLS1 protein that has been treated with the test compound to the activity of an untreated LLS1 protein, thereby selecting compounds with potential for inhibitory activity.

Figure 1 (page 1 of 3)

```
SEQ ID NO:18  ------------------------------------------------------------
SEQ ID NO:22  TKTSAPFLFTLSLRSNMTEERNVRKTRV-------VDVVLDCVIPYIDDPKDRDAVSQVC
SEQ ID NO:20  MGGEAP------------EARRLDRAMSFGGAGSIPEEALHLVLGYVDDPRDREAVSLVC
SEQ ID NO:37  M-----------------EDPDIKRCKL--SCVATVDDVIEQVMTYITDPKDRDSASLVC
              1                                                          60

SEQ ID NO:18  ----------------------------------------TRPRT--------------
SEQ ID NO:22  RRWYELDSLTRKHVTIALCYTTTPARLRRRFPHLESLKLKGKPRAAMFNLIPEDWGGHVT
SEQ ID NO:20  RRWHRIDALTRKHVTVPFCYAASPAHLLARFPRLESLAVKGKPRAAMYGLIPEDWGAYAR
SEQ ID NO:37  RRWFKIDSETREHVTMALCYTATPDRLSRRFPNLRSLKLKGKPRAAMFNLIPENWGGYVT
              61                                                         120

SEQ ID NO:18  ------------------------------------------------------------
SEQ ID NO:22  PWVKEISQYFDCLKSLHFRRMIVKDSDLQNLARDRGHVLHALKLDKCSGFTTDGLFHIGR
SEQ ID NO:20  PWVAELAAPLECLKALHLRRMVVTDDDLAALVRARGHMLQELKLDKCSGFSTDALRLVAR
SEQ ID NO:37  PWVTEISNNLRQLKSVHFRRMIVSDLDLDRLAKARADDLETLKLDKCSGFTTDGLLSIVT
              121                                                        180

                     *  **          *   **  *  ** ***  *         **     *
SEQ ID NO:18  --RGLETLFLEESTIDEKENDEWIRELATSNSVLETLNFFLTDL-RASPEYLTLLVRNCQ
SEQ ID NO:22  FCKSLRVLFLEESSILEKD-GEWLHELALNNTVLETLNFYLTDIAVVKIEDLELLAKNCP
SEQ ID NO:20  SCRSLRTLFLEECSIADNGT-EWLHDLAVNNPVLETLNFHMTEL-TVVPADLELLAKKCK
SEQ ID NO:37  HCRKIKTLLMEESSFSEKD-GKWLHELAQHNTSLEVLNFYMTEFAKISPKDLETIARNCR
              181                                                        240
```

Figure 1 (page 2 of 3)

```
                    *        *   *  *  * ** **   *          *     *  * ** *
SEQ ID NO:18  RLKTLKISECFMPDLVSLFRTAQTLQEFAGGSFEEQGQPVASRNYENYYFPPSLHRLSLL
SEQ ID NO:22  NLVSVKLTDCEILDLVNFFKHASALEEFCGGTYNE--E---PERYSAISLPAKLCRLGLT
SEQ ID NO:20  SLISLKISDCDFSDLIGFFRMAASLQEFAGGAFIEQGELT---KYGNVKFPSRLCSLGLT
SEQ ID NO:37  SLVSVKVGDFEILELVGFFKAAANLEEFCGGSLNE--DIGMPEKYMNLVFPRKLCRLGLS
            241                                                         300

                  * * *   * *       ****    * *****     * **  *  * ******* *
SEQ ID NO:18  YMGTNDMQILFPYATALKKLDLQFTFLSTEDHCQIVQRCSNLETLEVRDVIGDRGLQVVA
SEQ ID NO:22  YIGKNELPIVFMFAAVLKKLDLLYAMLDTEDHCMLIQRCPNLEVLETRNVIGDRGLEVLG
SEQ ID NO:20  YMGTNEMPIIFPFSALLKKLDLQYTFLTTEDHCQLIAKCPNLLVLAVRNVIGDRGLGVVA
SEQ ID NO:37  YMGPNEMPILFPFAAQIRKLDLLYALLETEDHCTLIQKCPNLEVLETRNVIGDRGLEVLA
            301                                                         360

                    ** * *** *** *  *    * *  *  **     ** ** * *  ******  **
SEQ ID NO:18  QTCKKLHRLRVERGDDDQGGLEDEQGRISQVGLMAIAQGCPELTYWAIHVSDITNAALEA
SEQ ID NO:22  RCCKRLKRLRIERGDDDQG-MEDEEGTVSHRGLIALSQGCSELEYMAVYVSDITNASLEH
SEQ ID NO:20  DTCKKLQRLRVERGDDDPG-LQEEQGGVSQVGLTTVAVGCRELEYIAAYVSDITNGALES
SEQ ID NO:37  QYCKQLKRLRIERGADEQG-MEDEEGLVSQRGLIALAQGCQELEYMAVYVSDITNESLES
            361                                                         420

                   **  *** ******** *  ** ******** ** ** ****** * * * * * ** *
SEQ ID NO:18  VGTCSKNLNDFRLVLLDREAHITELPLDNGVRALLRGCTKLRRFAFYVRPGALSDVGLGY
SEQ ID NO:22  IGTHLKNLCDFRLVLLDHEEKITDLPLDNGVRALLRGCDKLRRFALYLRRGGLTDVGLGY
SEQ ID NO:20  IGTFCKNLCDFRLVLLDREERITDLPLDNGVRALLRGCTKLRRFALYLRPGGLSDTGLGY
SEQ ID NO:37  IGTYLKNLCDFRLVLLDREERITDLPLDNGVRSLLIGCKKLRRFAFYLRQGGLTDLGLSY
            421                                                         480
```

Figure 1 (page 3 of 3)

```
                 *  *     **** *** * *      **  * *** * *  *** * * *     ***
SEQ ID NO:18  VGEFSKSIRYMLLGNVGESDNGIIQLSKGCPSLQKLEVRGC-LFSEHALALAALQLKSLR
SEQ ID NO:22  IGQYSPNVRWMLLGYVGESDAGLLEFAKGCPSLQKLEMRGCLFFSERALAVAATQLTSLR
SEQ ID NO:20  IGQYSGIIQYMLLGNVGETDDGLIRFALGCENLRKLELRSC-CFSEQALARAIRSMPSLR
SEQ ID NO:37  IGQYSPNVRWMLLGYVGESDEGLMEFSRGCPNLQKLEMRGC-CFSERAIAAAVTKLPSLR
            481                                                         540

                 ******   *  * *   * ** ****   *                      ********
SEQ ID NO:18  YLWVQGFRSSPTGTDIMAMVRPFWNIEYIVP---------DQDEPCPEHKRQILAYYSLA
SEQ ID NO:22  YLWVQGYGVSPSGRDLLVMARPFWNIELI-PSRKVATNTNPDETVVVEHPAHILAYYSLA
SEQ ID NO:20  YVWVQGYKASKTGHDLMLMARPFWNIEFTPPSSENANRMREDGEPCVDSQAQILAYYSLA
SEQ ID NO:37  YLWVQGYRASMTGQDLMQMARPYWNIELI-PSRRVPEVNQQGEIREMEHPAHILAYYSLA
            541                                                         600

                 * * * *  *  *
SEQ ID NO:18  GRRTDCPPSVTLLYPAF----
SEQ ID NO:22  GQRSDFPDTVVPLDTATCVDT
SEQ ID NO:20  GKRSDCPRSVVPLYPA-----
SEQ ID NO:37  GQRTDCPTTVRVLKEPI----
            601                   621
```

Figure 2 (page 1 of 3)

```
                                                                         ***
SEQ ID NO:30    MPVMAPTASL---LLSPRPLPASRRVPSLPA--------LSASGRLRLRRARADTRLRVA
SEQ ID NO:32    MALPHSISALATTLTLSSPITKPHKVNPFPFSSNRNSQFLTKQTRPRSRRNLSLTPARVA
SEQ ID NO:36    --------------------------------------------------------LRVA
SEQ ID NO:38    MRATIPALSL---LVTPR-------LPSLAV--------PLAGGRLR-EGGRSRTRLRVA
                1                                                         60

                ** *                          ** ********** *** *  ******* *
SEQ ID NO:30    APPSVPGEAD--QAPGETEPSTSSAD---EKFVWRDHWYPVSLVEDLDPSVPTPFQLLNR
SEQ ID NO:32    APPSTVEADRLYPEAENNETEEEFSDESSSKFTWRDHWYPVSLIEDLNPLLPTPFQLLGR
SEQ ID NO:36    APTSVPGEAE--RAEEPSTSTSTSPESSGEKFVWRDHWYPVSLVEDLDPRVPTPFQLLNR
SEQ ID NO:38    APTSVPGEAA--EQAEPSTSAPES----GEKFSWRDHWYPVSLVEDLDPSRPTPFQLLNR
                61                                                       120

                  * *       *** ** *************** * ************ * *  ****
SEQ ID NO:30    DLVIWKDPKSGEWVALDDRCPHRLAPLSEGRIDETGCLQCSYHGWSFDGSGACTRIPQAA
SEQ ID NO:32    EIVLWYDKSISQWVAFDDKCPHRLAPLSEGRIDEDGKLQCSYHGWSFDGCGSCVKIPQAS
SEQ ID NO:36    DLVIWNDPNSGDWVALDDRCPHRLAPLSEGRIDETGGLQCSYHGWSFDGSGACTRIPQAA
SEQ ID NO:38    DLVIWKEPKSGEWVALDDRCPHRLAPLSEGRIDETGCLQCSYHGWSFDGSGACTKIPQAM
                121                                                      180

                 ***** *  ** ***  **** ******** ***** ** *  *** *  *  * * **
SEQ ID NO:30    PEGPEAKAVRSPKACAIKFPTLVSQGLLFVWPDENGWEKATATKPPMLPKEFEDPAFSTV
SEQ ID NO:32    SEGPEARAIGSPKACATRFPTLVSQGLLFVWADENGWEKAKASNPPMFPDDFDKPEFPTV
SEQ ID NO:36    PEGPEARAVRSPRACATKFPTLLSQGLLFVWPDENGWDKAKATKPPMLPKEFDDPAFSTV
SEQ ID NO:38    PEGPEARAVRSPKACAIKFPTLVSQGLLFVWPDENGWEKAAATKPPMLPKEFEDPAFSTV
                181                                                      240
```

Figure 2 (page 2 of 3)

```
                 ***** ******************** ********** ** * * *** ** ** * ***
SEQ ID NO:30  TIQRDLYYGYDTLMENVSDPSHIEFAXHKVTGRRDRARPLPFKMESSGAWGYSGSNSGNP
SEQ ID NO:32  NIQRDLFYGYDTLMENVSDPSHIEFAHHKVTGRRDRAKPLPFKMDSRGSWGFSGANEGNP
SEQ ID NO:36  TIQRDLFYGYDTLMENVSDPSHIEFAHHKVTGRRDRAKPLPFKMESSGAWGYSGANTGNP
SEQ ID NO:38  TIQRDLFYGYDTLMENVSDPSHIEFAHHKVTGRRDRARPLTFRMESSGAWGYSGANSGNP
            241                                                         300

                 * * * ****  **********  ****** ****** ********************
SEQ ID NO:30  RISATFVAPCYALNKIEIDTKLPIFGDQKWVIWICSFNIPMAPGKTRSIVCSARNFFQFS
SEQ ID NO:32  QISAKFVAPCYMMNKIEIDTKLPVVGDQKWVVWICSFNVPMAPGKTRSIVCSARNFFQFS
SEQ ID NO:36  RITATFEAPCYALNKIEIDTKLPIVGDQKWVIWICSFNIPMAPGKTRSIVCSARNFFQFT
SEQ ID NO:38  RITATFEAPCYALNKIEIDTKLPIFGDQKWVIWICSFNIPMAPGKTRSIVCSARNFFQFT
            301                                                         360

                 ** ****                      ********** ************** ** *
SEQ ID NO:30  MPGKAWWQL-------------------VPRWYEHWTSNLVYDGDMIVLQGQEKIFLSAS
SEQ ID NO:32  VPGPAWWQVNVILLFAFNFKQCIHVTQVVPRWYEHWTSNKVYDGDMIVLQGQEKIFLSET
SEQ ID NO:36  MPGKAWWQF-------------------VPRWYEHWTSNLVYDGDMIVLQGQEKVFLSAS
SEQ ID NO:38  MPGKAWWQL-------------------VPRWYEHWTSNLVYDGDMIVLQGQEKIFLAAT
            361                                                         420

                 **   * * ***  ************ * ***  ** ** * *      *********
SEQ ID NO:30  KESSADINQQYTKITFTPTQADRFVLAFRAWLRKFGNSQPDWFGNPSQEVLPSTVLSKRE
SEQ ID NO:32  KEGG-DINKQYTNITFTPTQADRFVLAFRNWLRRHGNGQPEWFGNSSDQPLPSTVLSKRQ
SEQ ID NO:36  KESSADVNQQYTKLTFTPTQADRFVLAFRAWLRKFGNSQPDWYGSPSQDALPSTVLSKRE
SEQ ID NO:38  KESSTDINQQYTKITFTPTQADRFVLACRTWLRKFGNSQPEWFGNPTQEALPSTVLSKRE
            421                                                         480
```

Figure 2 (page 3 of 3)

```
                 **** **  ******  *   **  ***  ****
SEQ ID NO:30  MLDRYEQHTLKCSSCKGAYNAFQTLQKVFMGATVA----------VLLLL--------
SEQ ID NO:32  MLDRFEQHTLKCSSCKAAYEGFQTWQKVLIGATVVFCATSGIPSDFQLRVLLAGLAVVSA
SEQ ID NO:36  MLDRYEQHTLKCSSCRGAHKAFQTLQKVFMGATVVFGATSGIPADVQLRILLGAGALVSA
SEQ ID NO:38  MLDRYEQLSLKCSSCKGAYNAFQNLQKVFMGATVVCCAAAGIPPDVQLRLLIGAAALVSA
              481                                                       540

SEQ ID NO:30  ------------------------
SEQ ID NO:32  AIAFALNQLQKNFEFVDYVHAEID
SEQ ID NO:36  ALAYVFYDRQKHFVFVDYVHADID
SEQ ID NO:38  AIAYAFHELQKNFVFVDYVHADID
              541                    564
```

SEQUENCE LISTING

<110> E. I. du Pont de Nemours and Company

<120> Disease Resistance Factors

<130> BB1356

<140>
<141>

<150> 60/133,041
<151> 1999-05-07

<160> 38

<170> Microsoft Office 97

<210> 1
<211> 701
<212> DNA
<213> Zea mays

<220>
<221> unsure
<222> (389)

<220>
<221> unsure
<222> (457)

<220>
<221> unsure
<222> (531)

<220>
<221> unsure
<222> (591)

<220>
<221> unsure
<222> (637)

<220>
<221> unsure
<222> (658)

<220>
<221> unsure
<222> (693)

<220>
<221> unsure
<222> (699)..(700)

<400> 1
gcggacgcgt gggctggaaa cacttttcct ggaagaaagc acaattgatg agaaagaaaa 60
tgatgagtgg atccgtgagc ttgctacgag caattctgtt cttgagacac tgaatttctt 120
tctaacagat ctcagggcat ccccagagta tcttaccctc cttgtgcgca actgtcaacg 180
attgaaaact ctgaagatta gtgaatgttt catgcccgat ctggtcagtt tgttccgaac 240

tgcacaaaca ctacaagagt tcgctggtgg ttcctttgaa gagcagggtc aacctgtggc 300
aagtagaaat tatgagaact actattttcc tccttcactg caccgcttga gtttgctcta 360
catgggaaca aatgatatgc aaatactgnt tccatatgct actgcactta agaagttaga 420
ccttcagttt acattccttt ccacagagga tcattgncag atagttcaac gctgctccaa 480
tctggaaacc ttagaggtga gggatgtcat aggggatcgg ggactacaag ntggtgcaca 540
gacctgcaag aaattgcata ggctcagagt agagagagga gatgatgatc nagaggtctt 600
gaggatgaac caaggtagga atttcacagg gtggggntga tgggtatagg cccaaggntg 660
gccttgggtt gacatactgg gccgatacca tgnattagnn c 701

<210> 2
<211> 194
<212> PRT
<213> Zea mays

<220>
<221> UNSURE
<222> (128)

<220>
<221> UNSURE
<222> (150)

<220>
<221> UNSURE
<222> (175)

<400> 2

Arg Gly Leu Glu Thr Leu Phe Leu Glu Glu Ser Thr Ile Asp Glu Lys
1 5 10 15

Glu Asn Asp Glu Trp Ile Arg Glu Leu Ala Thr Ser Asn Ser Val Leu
20 25 30

Glu Thr Leu Asn Phe Phe Leu Thr Asp Leu Arg Ala Ser Pro Glu Tyr
35 40 45

Leu Thr Leu Leu Val Arg Asn Cys Gln Arg Leu Lys Thr Leu Lys Ile
50 55 60

Ser Glu Cys Phe Met Pro Asp Leu Val Ser Leu Phe Arg Thr Ala Gln
65 70 75 80

Thr Leu Gln Glu Phe Ala Gly Gly Ser Phe Glu Glu Gln Gly Gln Pro
85 90 95

Val Ala Ser Arg Asn Tyr Glu Asn Tyr Tyr Phe Pro Pro Ser Leu His
100 105 110

Arg Leu Ser Leu Leu Tyr Met Gly Thr Asn Asp Met Gln Ile Leu Xaa
115 120 125

Pro Tyr Ala Thr Ala Leu Lys Lys Leu Asp Leu Gln Phe Thr Phe Leu
130 135 140

Ser Thr Glu Asp His Xaa Gln Ile Val Gln Arg Cys Ser Asn Leu Glu
145 150 155 160

Thr Leu Glu Val Arg Asp Val Ile Gly Asp Arg Gly Leu Gln Xaa Gly
165 170 175

Ala Gln Thr Cys Lys Lys Leu His Arg Leu Arg Val Glu Arg Gly Asp
180 185 190

Asp Asp

<210> 3
<211> 844
<212> DNA
<213> Oryza sativa

<220>
<221> unsure
<222> (352)

<220>
<221> unsure
<222> (823)

<400> 3
atcactccaa cttactagtt cttgcggtga ggaatgtgat tggagataga ggattagggg 60
ttgttgcaga cacatgcaag aagctacaaa gactcagagt tgagcgagga gatgatgatc 120
caggtttgca agaagaacaa ggaggagtct ctcaagtcgg gttgacaact gtagccgtag 180
gatgccgtga actggaatac atagctgcct atgtgtctga tatcacaaat ggggccctgg 240
agtctattgg gactttctgc aaaaatcttt gcgacttccg tcttgtccta ctcgatagag 300
aagagaggat aacagatttg cccttagaca atggtgtccg tgcactgctg angggctgca 360
cgaaacttcg gaggtttgct ctatacttga gaccaggggg actttcagat acaggccttg 420
gctatattgg acagtacagt ggaattatcc aatacatgct tctgggtaat gttggggaaa 480
cagatgatgg tctgatccgg tttgcattgg ggtgtgagaa cctgcggaag cttgagctaa 540
ggagttgttg cttcagtgag caagctttag cccgcgctat acggagtatg ccttccctga 600
gatacgtgtg ggtacagggc tacaaggctt ctaagactgg tcacgatctc atgctcatgg 660
caggcccttc tggaacatag agtttacacc tcccagaaga ctggtcacga tctcatgctc 720
atggcaggcc cttctggaac atagagttta cacctcccag ttctgagaat gcaaatcgaa 780
tgagagaaga tggtgaacct tgtgtaatat caactcagat acnctgcgga cgtaatacga 840
tagg 844

<210> 4
<211> 236
<212> PRT
<213> Oryza sativa

<220>
<221> UNSURE
<222> (115)

<400> 4
Asn Leu Leu Val Leu Ala Val Arg Asn Val Ile Gly Asp Arg Gly Leu
1 5 10 15

Gly Val Val Ala Asp Thr Cys Lys Lys Leu Gln Arg Leu Arg Val Glu
20 25 30

Arg Gly Asp Asp Asp Pro Gly Leu Gln Glu Glu Gln Gly Gly Val Ser
35 40 45

Gln Val Gly Leu Thr Thr Val Ala Val Gly Cys Arg Glu Leu Glu Tyr
50 55 60

Ile Ala Ala Tyr Val Ser Asp Ile Thr Asn Gly Ala Leu Glu Ser Ile
65 70 75 80

Gly Thr Phe Cys Lys Asn Leu Cys Asp Phe Arg Leu Val Leu Leu Asp
85 90 95

Arg Glu Glu Arg Ile Thr Asp Leu Pro Leu Asp Asn Gly Val Arg Ala
100 105 110

Leu Leu Xaa Gly Cys Thr Lys Leu Arg Arg Phe Ala Leu Tyr Leu Arg
115 120 125

Pro Gly Gly Leu Ser Asp Thr Gly Leu Gly Tyr Ile Gly Gln Tyr Ser
130 135 140

Gly Ile Ile Gln Tyr Met Leu Leu Gly Asn Val Gly Glu Thr Asp Asp
145 150 155 160

Gly Leu Ile Arg Phe Ala Leu Gly Cys Glu Asn Leu Arg Lys Leu Glu
165 170 175

Leu Arg Ser Cys Cys Phe Ser Glu Gln Ala Leu Ala Arg Ala Ile Arg
180 185 190

Ser Met Pro Ser Leu Arg Tyr Val Trp Val Gln Gly Tyr Lys Ala Ser
195 200 205

Lys Thr Gly His Asp Leu Met Leu Met Ala Arg Pro Phe Trp Asn Ile
210 215 220

Glu Phe Thr Pro Pro Arg Arg Leu Val Thr Ile Ser
225 230 235

<210> 5
<211> 482
<212> DNA
<213> Glycine max

<400> 5
gcggaagaca cgtgtggtcg acgtggtcct cgactgcgtc atcccttaca tcgacgaccc 60
caaggaccgc gacgccgttt cccaggtgtg tcgacgctgg tacgagctcg actcgctcac 120
ccgcaagcac gtcaccatcg cgctctgcta caccaccacc ccggctcgcc tccgccgccg 180
cttcccgcac ctcgagtcgc tcaagctcaa gggcaagccc cgagccgcaa tgttcaactt 240
gatacccgag gattggggcg gacacgtcac tccctgggtc aaagagattt ctcaagtact 300
tcgattgcct caagagcctc cacttccgcc gcatgattgt caagggattc cgatcttcag 360
aatctcgctc gtgaccgcgg tcacgtgctt cacgctctca aagcttgaca agtgctccgg 420
tttcaacaac gatggtcctt tccatatcgg gtcgcttttg caaagaagtt taagagtcct 480
gt 482

<210> 6
<211> 108
<212> PRT
<213> Glycine max

<220>
<221> UNSURE
<222> (97)

<400> 6
Val Asp Val Val Leu Asp Cys Val Ile Pro Tyr Ile Asp Asp Pro Lys
1 5 10 15

Asp Arg Asp Ala Val Ser Gln Val Cys Arg Arg Trp Tyr Glu Leu Asp
20 25 30

Ser Leu Thr Arg Lys His Val Thr Ile Ala Leu Cys Tyr Thr Thr Thr
35 40 45

Pro Ala Arg Leu Arg Arg Arg Phe Pro His Leu Glu Ser Leu Lys Leu
50 55 60

Lys Gly Lys Pro Arg Ala Ala Met Phe Asn Leu Ile Pro Glu Asp Trp
65 70 75 80

Gly Gly His Val Thr Pro Trp Val Lys Glu Ile Ser Gln Val Leu Arg
85 90 95

Xaa Leu Lys Ser Leu His Phe Arg Arg Met Ile Val
100 105

<210> 7
<211> 794
<212> DNA
<213> Triticum aestivum

<220>
<221> unsure
<222> (270)

<220>
<221> unsure
<222> (356)

<220>
<221> unsure
<222> (675)

<220>
<221> unsure
<222> (689)

<220>
<221> unsure
<222> (702)

<220>
<221> unsure
<222> (729)

<220>
<221> unsure
<222> (743)

<220>
<221> unsure
<222> (752)

<220>
<221> unsure
<222> (761)

<220>
<221> unsure
<222> (769)

<220>
<221> unsure
<222> (777)

<220>
<221> unsure
<222> (783)

<220>
<221> unsure
<222> (785)

<220>
<221> unsure
<222> (790)

<220>
<221> unsure
<222> (793)

<400> 7
gtaggattga tggctgtagc tgaaggctgt cctgatttgg agtactgggc agtacatgtg 60
tctgacatta caaatgcagc tcttgaggct attggcgcat tcagcaaaaa cctgaacgat 120
ttccgacttg tcctgcttga tagagaggtg catataactg aactgcccct tgacaacggg 180
gttcgggctt tgctgagagg ttgcaccaaa ctccggaggt ttgcatttta tgtgagacct 240
ggagctctat cagatattgg cctttcttan gttgggcgaa tttagcaaga ccgtccgcta 300
catgttgctt gggaatgccg gggggtctga tgatggactg ctggcatttg cacgangatg 360
cccaagcttg cagaaattgg agctaaggag ttgctgcttt agtgaacgtg cattggcagt 420
tgcagcctta cagctgaagt cactcagata tctttgggtg cagggataca aggcatctcc 480
tactggcacc gatctcatgg caatggtacg ccccttctgg aacattgagt ttattgcacc 540
aaatcaagat gagccttgcc cagagggtca ggacagattt ggcatactac tctctggtgg 600
ggaaggcaga ttgtcctagt cagtattccc tccatcgtag tgggagctaa aagaccacca 660
ccagtttact gacancatgt tgatgcagna accacatcgg anaggaattc actacagtgc 720
aattagggnt gaagctcagt aangaccatc tnatgcttga nttagggana tttgggnact 780
gtnantgcan agna 794

<210> 8
<211> 177
<212> PRT
<213> Triticum aestivum

<220>
<221> UNSURE
<222> (89)

<220>
<221> UNSURE
<222> (118)

<400> 8
Gly Leu Met Ala Val Ala Glu Gly Cys Pro Asp Leu Glu Tyr Trp Ala
1 5 10 15

Val His Val Ser Asp Ile Thr Asn Ala Ala Leu Glu Ala Ile Gly Ala
20 25 30

```
Phe Ser Lys Asn Leu Asn Asp Phe Arg Leu Val Leu Leu Asp Arg Glu
         35                  40                  45

Val His Ile Thr Glu Leu Pro Leu Asp Asn Gly Val Arg Ala Leu Leu
     50                  55                  60

Arg Gly Cys Thr Lys Leu Arg Arg Phe Ala Phe Tyr Val Arg Pro Gly
 65                  70                  75                  80

Ala Leu Ser Asp Leu Ala Phe Leu Xaa Leu Gly Glu Phe Ser Lys Thr
                 85                  90                  95

Val Arg Tyr Met Leu Leu Gly Asn Ala Gly Gly Ser Asp Asp Gly Leu
            100                 105                 110

Leu Ala Phe Ala Arg Xaa Cys Pro Ser Leu Gln Lys Leu Glu Leu Arg
        115                 120                 125

Ser Cys Cys Phe Ser Glu Arg Ala Leu Ala Val Ala Ala Leu Gln Leu
    130                 135                 140

Lys Ser Leu Arg Tyr Leu Trp Val Gln Gly Tyr Lys Ala Ser Pro Thr
145                 150                 155                 160

Gly Thr Asp Leu Met Ala Met Val Arg Pro Phe Trp Asn Ile Glu Phe
                165                 170                 175

Ile
```

<210> 9
<211> 426
<212> DNA
<213> Oryza sativa

<220>
<221> unsure
<222> (270)

<220>
<221> unsure
<222> (380)

<220>
<221> unsure
<222> (393)

<220>
<221> unsure
<222> (396)..(397)

<220>
<221> unsure
<222> (408)

<220>
<221> unsure
<222> (413)

<220>
<221> unsure
<222> (418)

<220>
<221> unsure
<222> (423)

<400> 9
gctgtgaggt cgccgaaggc gtgcgcgatc aagttcccca ccctcgtctc gcaagggctg 60
ctcttcgtgt ggcccgacga gaatgggtgg gagaaggcca cggctaccaa gcctccgatg 120
ttaccgaagg agtttgagga tcctgcgttc tccacggtga ccatccagag ggatctgtac 180
tatggctatg atacattgat ggagaacgtc tctgatccgt cgcatataga atttgctcac 240
cacaaggtca ctgggtcgaa gagatcgaan caagcctttt gccaattcaa gaatgggaat 300
caaagttggt gcaatggggg ataattcaag gggtcaaatt tctgggaaaa ccctccgcat 360
caagtggcaa ctttttttgtn ggccccccttg ccnatnncac ttgaaacnaa aanttggnga 420
atnaga 426

<210> 10
<211> 107
<212> PRT
<213> Oryza sativa

<220>
<221> UNSURE
<222> (90)

<400> 10
Ala Val Arg Ser Pro Lys Ala Cys Ala Ile Lys Phe Pro Thr Leu Val
1 5 10 15

Ser Gln Gly Leu Leu Phe Val Trp Pro Asp Glu Asn Gly Trp Glu Lys
20 25 30

Ala Thr Ala Thr Lys Pro Pro Met Leu Pro Lys Glu Phe Glu Asp Pro
35 40 45

Ala Phe Ser Thr Val Thr Ile Gln Arg Asp Leu Tyr Tyr Gly Tyr Asp
50 55 60

Thr Leu Met Glu Asn Val Ser Asp Pro Ser His Ile Glu Phe Ala His
65 70 75 80

His Lys Val Thr Gly Ser Lys Arg Ser Xaa Gln Ala Phe Cys Gln Phe
85 90 95

Lys Asn Gly Asn Gln Ser Trp Cys Asn Gly Gly
100 105

<210> 11
<211> 465
<212> DNA
<213> Glycine max

<220>
<221> unsure
<222> (460)

<400> 11
aaaccattga tggcgctccc tcactccatc tctgccttag ccaccacact cacactctcc 60
tccccaataa ccaaacccca taaagttaac ccctttccct tttcctcgaa ccgaaattca 120
caatttttaa cgaaacaaac gcgacccaga agcagaagaa acctctccct aacccctgca 180
cgcgttgcgg cgccaccctc aacggttgaa gccgatcgat tatacccaga ggccgaaaat 240
aacgaaactg aggaagagtt tagcgacgag agctcttcct ctaaattcac ttggagggat 300
cactggtacc ctgtctcgtt aattgaagat ctgaaccctc tcttgcccac accgtttcag 360
cttctgggtc gtgaaatcgt gctctggtac gacaagtcca tttcccaatg ggttgctttt 420
gatgacaaat gcccccatcg tcttgccccc ttatctgaan ggagg 465

<210> 12
<211> 66
<212> PRT
<213> Glycine max

<220>
<221> UNSURE
<222> (65)

<400> 12
Glu Ser Ser Ser Ser Lys Phe Thr Trp Arg Asp His Trp Tyr Pro Val
1 5 10 15

Ser Leu Ile Glu Asp Leu Asn Pro Leu Leu Pro Thr Pro Phe Gln Leu
20 25 30

Leu Gly Arg Glu Ile Val Leu Trp Tyr Asp Lys Ser Ile Ser Gln Trp
35 40 45

Val Ala Phe Asp Asp Lys Cys Pro His Arg Leu Ala Pro Leu Ser Glu
50 55 60

Xaa Arg
65

<210> 13
<211> 558
<212> DNA
<213> Triticum aestivum

<220>
<221> unsure
<222> (207)

<220>
<221> unsure
<222> (216)

<220>
<221> unsure
<222> (249)

<220>
<221> unsure
<222> (254)

<220>
<221> unsure
<222> (269)

<220>
<221> unsure
<222> (294)

<220>
<221> unsure
<222> (310)

<220>
<221> unsure
<222> (330)

<220>
<221> unsure
<222> (335)

<220>
<221> unsure
<222> (339)..(340)

<220>
<221> unsure
<222> (365)

<220>
<221> unsure
<222> (386)

<220>
<221> unsure
<222> (404)

<220>
<221> unsure
<222> (406)

<220>
<221> unsure
<222> (445)

<220>
<221> unsure
<222> (451)

<220>
<221> unsure
<222> (462)

<220>
<221> unsure
<222> (470)

<220>
<221> unsure
<222> (475)

<220>
<221> unsure
<222> (483)..(484)

<220>
<221> unsure
<222> (490)

<220>
<221> unsure
<222> (496)

<220>
<221> unsure
<222> (498)

<220>
<221> unsure
<222> (511)

<220>
<221> unsure
<222> (514)

<220>
<221> unsure
<222> (520)

<220>
<221> unsure
<222> (522)

<220>
<221> unsure
<222> (528)

<220>
<221> unsure
<222> (533)

<220>
<221> unsure
<222> (545)

<400> 13
ccagggcctg ctcttcgtct ggcctgacga gaatggatgg gacaaggcca aggccaccaa 60
gcctccaatg ctgccgaagg agttcgatga cccggccttc tccaccgtga cgatccagag 120
ggacctcttc tatgggtatg acacgttgat ggagaacgtc tctgatccct cgcatataga 180
atttgctcac cacaaggtca ctggacnaag agatanagcc aagcctttgc catttaaaat 240
ggaatcaant ggcncatggg gatattcang ggcaaatacc ggcaatcctc gcancactgc 300
aactttcgan gccccttggc tatgcactgn aacanaatnn agattgacac caaattaacc 360
gattntggga gatcacaaat gggtcntatg gatttgctcc ttcnanattc caaaggccca 420
aggaaaatcg ttctattgtc cgtantgctc naaacttttc antttaaatn ccacnaagga 480
tgnngaattn tccccnantg tacaacattg ngcncaattn gncatgangc aantatctct 540
tcagncacaa agttccgt 558

<210> 14
<211> 105
<212> PRT
<213> Triticum aestivum

<220>
<221> UNSURE
<222> (69)

<220>
<221> UNSURE
<222> (72)

<220>
<221> UNSURE
<222> (83)

<220>
<221> UNSURE
<222> (85)

<220>
<221> UNSURE
<222> (90)

<220>
<221> UNSURE
<222> (98)

<220>
<221> UNSURE
<222> (103)

<400> 14

```
Gln Gly Leu Leu Phe Val Trp Pro Asp Glu Asn Gly Trp Asp Lys Ala
  1               5                  10                  15

Lys Ala Thr Lys Pro Pro Met Leu Pro Lys Glu Phe Asp Asp Pro Ala
             20                  25                  30

Phe Ser Thr Val Thr Ile Gln Arg Asp Leu Phe Tyr Gly Tyr Asp Thr
         35                  40                  45

Leu Met Glu Asn Val Ser Asp Pro Ser His Ile Glu Phe Ala His His
     50                  55                  60

Lys Val Thr Gly Xaa Arg Asp Xaa Ala Lys Pro Leu Pro Phe Lys Met
 65                  70                  75                  80

Glu Ser Xaa Gly Xaa Trp Gly Tyr Ser Xaa Ala Asn Thr Gly Asn Pro
                 85                  90                  95

Arg Xaa Thr Ala Thr Phe Xaa Ala Pro
            100                 105
```

<210> 15
<211> 562
<212> DNA
<213> Zea mays

<220>
<221> unsure
<222> (136)

<220>
<221> unsure
<222> (562)

<400> 15
cgccgtgctc gtccgcgcgc gcgccacatg ctacaggtgc tcaagctcga caagtgctcc 60
ggcttctcaa cggacgccct ccgcctcgtc gcccgctcct gcagatctct gagaactttg 120
ttcctggaag aatgtntaat tgccgatgaa gggagcgaat ggctccatga actcgccgtc 180
aacaattctg ttctggtgac actgaacttc tacatgacag aactcaaagt ggagcctgcc 240
gatctggagc ttcttgcaag gaactgtaaa tcattgattt ctctgaagat gagtgactgc 300
gatctttcgg atttgatggt tttctccaaa cctccaaggc actgcaagaa ttcgctggag 360
gcgctttttt cgaaatcgga gagtacacca agtacgaaaa ggtcaagctc ccacctaagc 420
tatgcttctt gggggggtctt accttcatgg gtaaaaacga gatgcccgtt aatctttccg 480
tattctgcgt tcgcttaaga aactggacct gcagtacact ttccctcacc actgaagatc 540
actgtcagct taatcgctaa an 562

<210> 16
<211> 186
<212> PRT
<213> Zea mays

<220>
<221> UNSURE
<222> (46)

<220>
<221> UNSURE
<222> (111)

<400> 16
Arg Arg Ala Arg Pro Arg Ala Arg His Met Leu Gln Val Leu Lys Leu
1 5 10 15

Asp Lys Cys Ser Gly Phe Ser Thr Asp Ala Leu Arg Leu Val Ala Arg
20 25 30

Ser Cys Arg Ser Leu Arg Thr Leu Phe Leu Glu Glu Cys Xaa Ile Ala
35 40 45

Asp Glu Gly Ser Glu Trp Leu His Glu Leu Ala Val Asn Asn Ser Val
50 55 60

Leu Val Thr Leu Asn Phe Tyr Met Thr Glu Leu Lys Val Glu Pro Ala
65 70 75 80

Asp Leu Glu Leu Leu Ala Arg Asn Cys Lys Ser Leu Ile Ser Leu Lys
85 90 95

Met Ser Asp Cys Asp Leu Ser Asp Leu Met Val Phe Ser Lys Xaa Ser
100 105 110

Lys Ala Leu Gln Glu Phe Ala Gly Gly Ala Phe Phe Glu Ile Gly Glu
115 120 125

Tyr Thr Lys Tyr Glu Lys Val Lys Leu Pro Pro Lys Leu Cys Phe Leu
130 135 140

Gly Gly Leu Thr Phe Met Gly Lys Asn Glu Met Pro Val Asn Leu Ser
145 150 155 160

Val Phe Cys Val Arg Leu Arg Asn Trp Thr Cys Ser Thr Leu Ser Leu
165 170 175

```
Thr Thr Glu Asp His Cys Gln Leu Asn Arg
            180                 185
```

<210> 17
<211> 1728
<212> DNA
<213> Zea mays

<400> 17

```
ccacgcgtcc gcggacgcgt gggctggaaa cacttttcct ggaagaaagc acaattgatg   60
agaaagaaaa tgatgagtgg atccgtgagc ttgctacgag caattctgtt cttgagacac  120
tgaatttctt tctaacagat ctcagggcat ccccagagta tcttaccctc cttgtgcgca  180
actgtcaacg attgaaaact ctgaagatta gtgaatgttt catgcccgat ctggtcagtt  240
tgttccgaac tgcacaaaca ctacaagagt tcgctggtgg ttcctttgaa gagcagggtc  300
aacctgtggc aagtagaaat tatgagaact actattttcc tccttcactg caccgcttga  360
gtttgctcta catgggaaca aatgatatgc aaatactgtt tccatatgct actgcactta  420
agaagttaga ccttcagttt acattccttt ccacagagga tcattgtcag atagttcaac  480
gctgctccaa tctggaaacc ttagaggtga gggatgtcat aggggatcgt ggactacaag  540
ttgttgcaca gacctgcaag aaattgcata ggctcagagt agagagagga gatgatgatc  600
aaggaggtct tgaggatgaa caaggtagga tttcacaggt ggggttgatg gctatagccc  660
aaggctgccc tgagttgaca tactgggcga tacatgtatc agacattaca aatgcagctt  720
tagaggcagt tggtacatgc agcaaaaatc ttaatgactt ccgccttgtc ctccttgata  780
gagaagcaca tataaccgaa ttgccactgg acaatggggt tcgtgctttg cttagaggtt  840
gcaccaaact acggaggttt gcattttatg tgagacctgg ggccctatct gatgttggtc  900
ttggctatgt tggagaattt agtaagagta ttcgttatat gttgcttggt aatgttggtg  960
aatctgataa tggaatcata caattatcaa aaggctgccc aagcttgcaa aaactggagg 1020
tgaggggttg tctctttagt gagcatgctt tagctttggc tgcactacag cttaagtcac 1080
tgaggtatct gtgggtacaa ggattcaggt catctccaac tggaactgat attatggcaa 1140
tggtacgccc cttctggaac attgagtata ttgttccaga tcaagatgaa ccttgcccag 1200
agcataagag acagattctg gcatactact cccttgctgg caggaggaca gattgtcctc 1260
catcagtaac tctgctttac ccggcatttt gagtgtagat acttgctttt tgccagactg 1320
aatctcatgg tactaagttc cattggtccc actatctgtg aagtaaatgg tccctgttct 1380
tccaattgat gaggacatgc agacgttcca gtgcaaagaa ccccaaaggt aagctttaag 1440
caggacggcc agctctgaac tgaggctagc tgagaacaat catgaatacc tgaaggcagc 1500
acttatgtca gcttggccta gctgtccagt atgggcatgt aagctttacc atcttttgta 1560
gttttggaga aacaattttg caataactac ccttgtttag tgtatattat cgattttcgt 1620
tcatatgctg ttgtattgtt gtattgaaca attatgtcaa ttaattagtc tacactctac 1680
agtctaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaag              1728
```

<210> 18
<211> 429
<212> PRT
<213> Zea mays

<400> 18

```
Thr Arg Pro Arg Thr Arg Gly Leu Glu Thr Leu Phe Leu Glu Glu Ser
  1               5                  10                  15

Thr Ile Asp Glu Lys Glu Asn Asp Glu Trp Ile Arg Glu Leu Ala Thr
             20                  25                  30

Ser Asn Ser Val Leu Glu Thr Leu Asn Phe Phe Leu Thr Asp Leu Arg
         35                  40                  45

Ala Ser Pro Glu Tyr Leu Thr Leu Leu Val Arg Asn Cys Gln Arg Leu
     50                  55                  60

Lys Thr Leu Lys Ile Ser Glu Cys Phe Met Pro Asp Leu Val Ser Leu
 65                  70                  75                  80
```

```
Phe Arg Thr Ala Gln Thr Leu Gln Glu Phe Ala Gly Gly Ser Phe Glu
                 85                  90                  95

Glu Gln Gly Gln Pro Val Ala Ser Arg Asn Tyr Glu Asn Tyr Tyr Phe
            100                 105                 110

Pro Pro Ser Leu His Arg Leu Ser Leu Leu Tyr Met Gly Thr Asn Asp
        115                 120                 125

Met Gln Ile Leu Phe Pro Tyr Ala Thr Ala Leu Lys Lys Leu Asp Leu
    130                 135                 140

Gln Phe Thr Phe Leu Ser Thr Glu Asp His Cys Gln Ile Val Gln Arg
145                 150                 155                 160

Cys Ser Asn Leu Glu Thr Leu Glu Val Arg Asp Val Ile Gly Asp Arg
                165                 170                 175

Gly Leu Gln Val Val Ala Gln Thr Cys Lys Lys Leu His Arg Leu Arg
            180                 185                 190

Val Glu Arg Gly Asp Asp Asp Gln Gly Gly Leu Glu Asp Glu Gln Gly
        195                 200                 205

Arg Ile Ser Gln Val Gly Leu Met Ala Ile Ala Gln Gly Cys Pro Glu
    210                 215                 220

Leu Thr Tyr Trp Ala Ile His Val Ser Asp Ile Thr Asn Ala Ala Leu
225                 230                 235                 240

Glu Ala Val Gly Thr Cys Ser Lys Asn Leu Asn Asp Phe Arg Leu Val
                245                 250                 255

Leu Leu Asp Arg Glu Ala His Ile Thr Glu Leu Pro Leu Asp Asn Gly
            260                 265                 270

Val Arg Ala Leu Leu Arg Gly Cys Thr Lys Leu Arg Arg Phe Ala Phe
        275                 280                 285

Tyr Val Arg Pro Gly Ala Leu Ser Asp Val Gly Leu Gly Tyr Val Gly
    290                 295                 300

Glu Phe Ser Lys Ser Ile Arg Tyr Met Leu Leu Gly Asn Val Gly Glu
305                 310                 315                 320

Ser Asp Asn Gly Ile Ile Gln Leu Ser Lys Gly Cys Pro Ser Leu Gln
                325                 330                 335

Lys Leu Glu Val Arg Gly Cys Leu Phe Ser Glu His Ala Leu Ala Leu
            340                 345                 350

Ala Ala Leu Gln Leu Lys Ser Leu Arg Tyr Leu Trp Val Gln Gly Phe
        355                 360                 365

Arg Ser Ser Pro Thr Gly Thr Asp Ile Met Ala Met Val Arg Pro Phe
    370                 375                 380

Trp Asn Ile Glu Tyr Ile Val Pro Asp Gln Asp Glu Pro Cys Pro Glu
385                 390                 395                 400
```

His Lys Arg Gln Ile Leu Ala Tyr Tyr Ser Leu Ala Gly Arg Arg Thr
405 410 415

Asp Cys Pro Pro Ser Val Thr Leu Leu Tyr Pro Ala Phe
420 425

<210> 19
<211> 2240
<212> DNA
<213> Oryza sativa

<400> 19
ccccccgggct gcaggaattc ggcacgagct ctcccttctc ctcctcttca ccaccaccac 60
caccaccagc agcagcagag agcaccatct ccatccaata atccccatgc ttgcgcacca 120
ctcccggcca catcccgcgc gaggaggagg aggaggagga gggtgtgctt gatccgcgct 180
cccgcctggt tggtggtggt ggggtgaggg gggagggatg ggaggggagg caccggaggc 240
gcggcggttg gaccgcgcga tgagcttcgg cggcgcgggc agcatcccgg aggaggcgct 300
gcacctggtg ctggggtacg tggacgaccc gcgggacagg gaggcggtgt cgctcgtgtg 360
ccgccgctgg caccgcatcg acgcgctcac gcggaagcac gtcaccgtgc ccttctgcta 420
cgccgcgtcg cccgcgcacc tgctcgcgcg gttcccgcgg ctggagtcgc tcgcggtcaa 480
ggggaagccg cgcgccgcca tgtacgggct catcccggag gactggggcg cctacgcgcg 540
cccctgggtc gccgagctcg ccgcgccgct cgagtgcctc aaggcgctcc acctgcgccg 600
catggtcgtc accgacgacg acctcgccgc gctcgtccgc gcccgcggcc acatgctgca 660
ggagctcaag ctcgacaagt gctccggctt ctccaccgac gctctccgcc tcgtcgcccg 720
ctcctgcaga tcactgagaa cattatttct ggaggaatgc tcaattgctg ataatggtac 780
tgaatggctc cacgaccttg ctgtcaacaa tcctgttctg gagacattga acttccacat 840
gaccgaactc acagtggtgc cagctgacct ggagcttctc gcaaagaagt gcaagtcact 900
aatttcattg aagatcagtg actgtgactt ttcagattta attggatttt tccggatggc 960
tgcatcattg caagagtttg cgggaggggc attcattgag caaggggagc tcactaagta 1020
tggaaatgtt aaattccctt caagactgtg ctccttagga cttacgtaca tggggacaaa 1080
cgagatgccc attatcttcc ctttctctgc attactcaag aagctggact tgcagtacac 1140
ttttctcacc actgaagatc actgccaact cattgcaaaa tgtcccaact tactagttct 1200
tgcggtgagg aatgtgattg gagatagagg attaggggtt gttgcagaca catgcaagaa 1260
gctacaaaga ctcagagttg agcgaggaga tgatgatcca ggtttgcaag aagaacaagg 1320
aggagtctct caagtcgggt tgacaactgt agccgtagga tgccgtgaac tggaatacat 1380
agctgcctat gtgtctgata tcacaaatgg ggccctggag tctattggga ctttctgcaa 1440
aaatctttgc gacttccgtc ttgtcctact cgatagagaa gagaggataa cagatttgcc 1500
cttagacaat ggtgtccgtg cactgctgag gggctgcacg aaacttcgga ggtttgctct 1560
atacttgaga ccaggggggac tttcagatac aggccttggc tatattggac agtacagtgg 1620
aattatccaa tacatgcttc tgggtaatgt tggggaaaca gatgatggtc tgatccggtt 1680
tgcattgggg tgtgagaacc tgcggaagct tgagctaagg agttgttgct tcagtgagca 1740
agctttagcc cgcgctatac ggagtatgcc ttccctgaga tacgtgtggg tacagggcta 1800
caaggcttct aagactggtc acgatctcat gctcatggcc aggcccttct ggaacataga 1860
gtttacacct cccagttctg agaatgcaaa tcgaatgaga gaagatggtg aaccttgtgt 1920
agatagtcaa gctcagatac ttgcatacta ctcccttgcc gggaagaggt cggactgccc 1980
acgatctgtg gttcctttgt atcctgcgtg actgtaaata ccgatatggt atctctctgc 2040
ttcgttcttg cctcttgcct tttttgggtg atatgttgat atgtggttat tgtatgggtc 2100
tagaactcta gatggctagc tgctatgtac sgtaataagc tactggtagc tgagatgtac 2160
tggaataagc acttctattt cccactctaa aaaaaaaaaa aaaaactcgg gcacgagggg 2220
gggcccggta cccaattcgc 2240

<210> 20
<211> 597
<212> PRT
<213> Oryza sativa

<400> 20
Met Gly Gly Glu Ala Pro Glu Ala Arg Arg Leu Asp Arg Ala Met Ser
1 5 10 15

```
Phe Gly Gly Ala Gly Ser Ile Pro Glu Glu Ala Leu His Leu Val Leu
            20                  25                  30

Gly Tyr Val Asp Asp Pro Arg Asp Arg Glu Ala Val Ser Leu Val Cys
        35                  40                  45

Arg Arg Trp His Arg Ile Asp Ala Leu Thr Arg Lys His Val Thr Val
    50                  55                  60

Pro Phe Cys Tyr Ala Ala Ser Pro Ala His Leu Leu Ala Arg Phe Pro
65                  70                  75                  80

Arg Leu Glu Ser Leu Ala Val Lys Gly Lys Pro Arg Ala Ala Met Tyr
                85                  90                  95

Gly Leu Ile Pro Glu Asp Trp Gly Ala Tyr Ala Arg Pro Trp Val Ala
            100                 105                 110

Glu Leu Ala Ala Pro Leu Glu Cys Leu Lys Ala Leu His Leu Arg Arg
        115                 120                 125

Met Val Val Thr Asp Asp Asp Leu Ala Ala Leu Val Arg Ala Arg Gly
    130                 135                 140

His Met Leu Gln Glu Leu Lys Leu Asp Lys Cys Ser Gly Phe Ser Thr
145                 150                 155                 160

Asp Ala Leu Arg Leu Val Ala Arg Ser Cys Arg Ser Leu Arg Thr Leu
                165                 170                 175

Phe Leu Glu Glu Cys Ser Ile Ala Asp Asn Gly Thr Glu Trp Leu His
            180                 185                 190

Asp Leu Ala Val Asn Asn Pro Val Leu Glu Thr Leu Asn Phe His Met
        195                 200                 205

Thr Glu Leu Thr Val Val Pro Ala Asp Leu Glu Leu Leu Ala Lys Lys
    210                 215                 220

Cys Lys Ser Leu Ile Ser Leu Lys Ile Ser Asp Cys Asp Phe Ser Asp
225                 230                 235                 240

Leu Ile Gly Phe Phe Arg Met Ala Ala Ser Leu Gln Glu Phe Ala Gly
                245                 250                 255

Gly Ala Phe Ile Glu Gln Gly Glu Leu Thr Lys Tyr Gly Asn Val Lys
            260                 265                 270

Phe Pro Ser Arg Leu Cys Ser Leu Gly Leu Thr Tyr Met Gly Thr Asn
        275                 280                 285

Glu Met Pro Ile Ile Phe Pro Phe Ser Ala Leu Leu Lys Lys Leu Asp
    290                 295                 300

Leu Gln Tyr Thr Phe Leu Thr Thr Glu Asp His Cys Gln Leu Ile Ala
305                 310                 315                 320

Lys Cys Pro Asn Leu Leu Val Leu Ala Val Arg Asn Val Ile Gly Asp
                325                 330                 335
```

Arg Gly Leu Gly Val Val Ala Asp Thr Cys Lys Lys Leu Gln Arg Leu
340 345 350

Arg Val Glu Arg Gly Asp Asp Asp Pro Gly Leu Gln Glu Glu Gln Gly
355 360 365

Gly Val Ser Gln Val Gly Leu Thr Thr Val Ala Val Gly Cys Arg Glu
370 375 380

Leu Glu Tyr Ile Ala Ala Tyr Val Ser Asp Ile Thr Asn Gly Ala Leu
385 390 395 400

Glu Ser Ile Gly Thr Phe Cys Lys Asn Leu Cys Asp Phe Arg Leu Val
405 410 415

Leu Leu Asp Arg Glu Glu Arg Ile Thr Asp Leu Pro Leu Asp Asn Gly
420 425 430

Val Arg Ala Leu Leu Arg Gly Cys Thr Lys Leu Arg Arg Phe Ala Leu
435 440 445

Tyr Leu Arg Pro Gly Gly Leu Ser Asp Thr Gly Leu Gly Tyr Ile Gly
450 455 460

Gln Tyr Ser Gly Ile Ile Gln Tyr Met Leu Leu Gly Asn Val Gly Glu
465 470 475 480

Thr Asp Asp Gly Leu Ile Arg Phe Ala Leu Gly Cys Glu Asn Leu Arg
485 490 495

Lys Leu Glu Leu Arg Ser Cys Cys Phe Ser Glu Gln Ala Leu Ala Arg
500 505 510

Ala Ile Arg Ser Met Pro Ser Leu Arg Tyr Val Trp Val Gln Gly Tyr
515 520 525

Lys Ala Ser Lys Thr Gly His Asp Leu Met Leu Met Ala Arg Pro Phe
530 535 540

Trp Asn Ile Glu Phe Thr Pro Pro Ser Ser Glu Asn Ala Asn Arg Met
545 550 555 560

Arg Glu Asp Gly Glu Pro Cys Val Asp Ser Gln Ala Gln Ile Leu Ala
565 570 575

Tyr Tyr Ser Leu Ala Gly Lys Arg Ser Asp Cys Pro Arg Ser Val Val
580 585 590

Pro Leu Tyr Pro Ala
595

<210> 21
<211> 2288
<212> DNA
<213> Glycine max

<400> 21
gcacgaggcc acacgttaca caggcgacta tggttgccgg aaacaaatcc ggatgggaaa 60
gggtgtatgt agctgttcct aggatgaata ttgtgataac agaacggcgt ttgaagcagt 120
gacgtgttac atcagtacat cacatcacat cacgtaaata taggtaataa gctcggaaaa 180

agttttgtcg tttcacaccc atctgtttgg ccctaccatt tcctcactca tcatccccat 240
aacccattcc ccttttgcca cttgaaccaa aacctctgca ccttttcttt tcactctcag 300
tctccgatcc aatatgacgg aggaacggaa cgtgcggaag acacgtgtgg tcgacgtggt 360
cctcgactgc gtcatccctt acatcgacga ccccaaggac cgcgacgccg tttcccaggt 420
gtgtcgacgc tggtacgagc tcgactcgct cacccgcaag cacgtcacca tcgcgctctg 480
ctacaccacc accccggctc gcctccgccg ccgcttcccg cacctcgagt cgctcaagct 540
caagggcaag ccccgagccg caatgttcaa cttgataccc gaggattggg gcggacacgt 600
cactccctgg gtcaaagaga tttctcagta cttcgattgc ctcaagagcc tccacttccg 660
ccgcatgatt gtcaaggatt ccgatcttca gaatctcgct cgtgaccgcg gtcacgtgct 720
tcacgctctc aagcttgaca agtgctccgg tttcaccacc gatggtcttt tccatatcgg 780
tcgcttttgc aagagtttaa gagtcttgtt tttggaggaa agctcaattc ttgagaagga 840
cggagaatgg ctacacgagc ttgctttgaa taatacagtt cttgagactc tcaattttta 900
cttgacagac attgctgttg tgaagattga ggaccttgaa cttttagcta aaaattgccc 960
caacttagtg tctgtgaaac ttactgactg tgaaatactg gatcttgtga acttctttaa 1020
gcatgcctct gcgctggaag agttttgtgg aggcacctac aacgaggaac cagaaagata 1080
ctctgctata tcattaccag caaagttatg tcgattgggt ttaacatata ttggaaagaa 1140
tgagttgccc attgtgttca tgtttgcagc cgtactaaaa aaattggatc tcctctatgc 1200
aatgctagac acggaggatc attgtatgtt aatccaaagg tgtccaaatc tggaagtcct 1260
tgagacaagg aatgtaattg gagatagagg gttagaggtt cttggtcgtt gttgtaagag 1320
gctaaaaagg cttaggattg aaaggggcga tgatgatcaa ggaatggagg atgaagaagg 1380
tactgtgtcc catagagggc taatagcctt gtcacagggc tgttcagagc ttgaatacat 1440
ggctgtttat gtgtctgata ttacaaatgc atctctggaa catattggaa ctcacttgaa 1500
gaacctctgt gattttcgcc ttgtgttgct tgaccatgaa gagaagataa ctgatttgcc 1560
acttgacaat ggggtgaggg ctctactgag gggctgtgac aagctgagga gatttgctct 1620
atatctcagg cgtggcgggt tgactgatgt aggccttggt tacattggac aatacagtcc 1680
aaatgtgaga tggatgctgc ttggttatgt gggggagtct gatgcagggc ttttggagtt 1740
cgctaagggg tgtcctagtc ttcagaaact tgaaatgaga gggtgtttat ttttcagtga 1800
acgtgcactt gctgtggctg caacacaatt gacttctctt aggtacttgt gggtgcaagg 1860
ttatggtgta tctccatctg gacgtgatct tttggtaatg gctcgaccct tttggaacat 1920
tgagttgatt ccttctagaa aggtggctac gaataccaat ccagatgaga ctgtagttgt 1980
tgagcatcct gctcatattc ttgcatatta ttctcttgca gggcagagat cagattttcc 2040
agatactgtt gtgcctttgg acactgccac atgcgttgat acctagaggc cagagctgtg 2100
tatatatacc agttttcttt tgtttttctt ctccccttc atatgctgtt tctatgttcc 2160
tgctctattt gtagttcatt ttagacaatt agtcttgtaa taagcctgtg ttttcatttg 2220
aaattctgaa acgcttcccc taacgctatt ggctccctta aaaactgaac attctcaatt 2280
ttgtgaat 2288

<210> 22
<211> 606
<212> PRT
<213> Glycine max

<400> 22
Thr Lys Thr Ser Ala Pro Phe Leu Phe Thr Leu Ser Leu Arg Ser Asn
1 5 10 15

Met Thr Glu Glu Arg Asn Val Arg Lys Thr Arg Val Val Asp Val Val
20 25 30

Leu Asp Cys Val Ile Pro Tyr Ile Asp Asp Pro Lys Asp Arg Asp Ala
35 40 45

Val Ser Gln Val Cys Arg Arg Trp Tyr Glu Leu Asp Ser Leu Thr Arg
50 55 60

Lys His Val Thr Ile Ala Leu Cys Tyr Thr Thr Thr Pro Ala Arg Leu
65 70 75 80

Arg Arg Arg Phe Pro His Leu Glu Ser Leu Lys Leu Lys Gly Lys Pro
85 90 95

```
Arg Ala Ala Met Phe Asn Leu Ile Pro Glu Asp Trp Gly Gly His Val
            100                 105                 110

Thr Pro Trp Val Lys Glu Ile Ser Gln Tyr Phe Asp Cys Leu Lys Ser
        115                 120                 125

Leu His Phe Arg Arg Met Ile Val Lys Asp Ser Asp Leu Gln Asn Leu
    130                 135                 140

Ala Arg Asp Arg Gly His Val Leu His Ala Leu Lys Leu Asp Lys Cys
145                 150                 155                 160

Ser Gly Phe Thr Thr Asp Gly Leu Phe His Ile Gly Arg Phe Cys Lys
                165                 170                 175

Ser Leu Arg Val Leu Phe Leu Glu Glu Ser Ser Ile Leu Glu Lys Asp
            180                 185                 190

Gly Glu Trp Leu His Glu Leu Ala Leu Asn Asn Thr Val Leu Glu Thr
        195                 200                 205

Leu Asn Phe Tyr Leu Thr Asp Ile Ala Val Val Lys Ile Glu Asp Leu
    210                 215                 220

Glu Leu Leu Ala Lys Asn Cys Pro Asn Leu Val Ser Val Lys Leu Thr
225                 230                 235                 240

Asp Cys Glu Ile Leu Asp Leu Val Asn Phe Phe Lys His Ala Ser Ala
                245                 250                 255

Leu Glu Glu Phe Cys Gly Gly Thr Tyr Asn Glu Glu Pro Glu Arg Tyr
            260                 265                 270

Ser Ala Ile Ser Leu Pro Ala Lys Leu Cys Arg Leu Gly Leu Thr Tyr
        275                 280                 285

Ile Gly Lys Asn Glu Leu Pro Ile Val Phe Met Phe Ala Ala Val Leu
    290                 295                 300

Lys Lys Leu Asp Leu Leu Tyr Ala Met Leu Asp Thr Glu Asp His Cys
305                 310                 315                 320

Met Leu Ile Gln Arg Cys Pro Asn Leu Glu Val Leu Glu Thr Arg Asn
                325                 330                 335

Val Ile Gly Asp Arg Gly Leu Glu Val Leu Gly Arg Cys Cys Lys Arg
            340                 345                 350

Leu Lys Arg Leu Arg Ile Glu Arg Gly Asp Asp Asp Gln Gly Met Glu
        355                 360                 365

Asp Glu Glu Gly Thr Val Ser His Arg Gly Leu Ile Ala Leu Ser Gln
    370                 375                 380

Gly Cys Ser Glu Leu Glu Tyr Met Ala Val Tyr Val Ser Asp Ile Thr
385                 390                 395                 400

Asn Ala Ser Leu Glu His Ile Gly Thr His Leu Lys Asn Leu Cys Asp
                405                 410                 415
```

Phe Arg Leu Val Leu Leu Asp His Glu Glu Lys Ile Thr Asp Leu Pro
420 425 430

Leu Asp Asn Gly Val Arg Ala Leu Leu Arg Gly Cys Asp Lys Leu Arg
435 440 445

Arg Phe Ala Leu Tyr Leu Arg Arg Gly Gly Leu Thr Asp Val Gly Leu
450 455 460

Gly Tyr Ile Gly Gln Tyr Ser Pro Asn Val Arg Trp Met Leu Leu Gly
465 470 475 480

Tyr Val Gly Glu Ser Asp Ala Gly Leu Leu Glu Phe Ala Lys Gly Cys
485 490 495

Pro Ser Leu Gln Lys Leu Glu Met Arg Gly Cys Leu Phe Phe Ser Glu
500 505 510

Arg Ala Leu Ala Val Ala Ala Thr Gln Leu Thr Ser Leu Arg Tyr Leu
515 520 525

Trp Val Gln Gly Tyr Gly Val Ser Pro Ser Gly Arg Asp Leu Leu Val
530 535 540

Met Ala Arg Pro Phe Trp Asn Ile Glu Leu Ile Pro Ser Arg Lys Val
545 550 555 560

Ala Thr Asn Thr Asn Pro Asp Glu Thr Val Val Val Glu His Pro Ala
565 570 575

His Ile Leu Ala Tyr Tyr Ser Leu Ala Gly Gln Arg Ser Asp Phe Pro
580 585 590

Asp Thr Val Val Pro Leu Asp Thr Ala Thr Cys Val Asp Thr
595 600 605

<210> 23
<211> 577
<212> DNA
<213> Triticum aestivum

<220>
<221> unsure
<222> (296)

<220>
<221> unsure
<222> (409)

<220>
<221> unsure
<222> (415)

<220>
<221> unsure
<222> (452)

<220>
<221> unsure
<222> (495)

<220>
<221> unsure
<222> (546)

<220>
<221> unsure
<222> (549)

<400> 23
gaactttgtt cctggaagaa tgtataattg ccgatgaagg gagcgaatgg ctccatgaac 60
tcgccgtcaa caattctgtt ctggtgacac tgaacttcta catgacagaa ctcaaagtgg 120
agcctgccga tctggagctt cttgcaagga actgtaaatc attgatttct ctgaagatga 180
gtgactgcga tctttcggat ttgattggtt ttctccaaac ctccaaggca ctgcaagaat 240
ccgctgggag gcgctttttt cgaagtcgga gagtacacca agtacgaaaa ggcaantccc 300
acctagctat gctcctgggg gggcctacct tcatgggtaa aaacgaatcc cgttactttc 360
cgtatccgcg tcgcttaaaa actggacctg catacacttc ctcacaacng aaatnacgtc 420
acttaacgct aaagcccaac ctacgggtct cnaggggggc cggtaccaat cgccctatat 480
gatcctatac cgcgncacgg gcgtccttta cactctgacg ggaaactggg taccactaac 540
cctganaanc ccttccactg gtatacaaag gccgacg 577

<210> 24
<211> 159
<212> PRT
<213> Triticum aestivum

<220>
<221> UNSURE
<222> (98)

<220>
<221> UNSURE
<222> (136)

<220>
<221> UNSURE
<222> (138)

<400> 24
Thr Leu Phe Leu Glu Glu Cys Ile Ile Ala Asp Glu Gly Ser Glu Trp
1 5 10 15

Leu His Glu Leu Ala Val Asn Asn Ser Val Leu Val Thr Leu Asn Phe
20 25 30

Tyr Met Thr Glu Leu Lys Val Glu Pro Ala Asp Leu Glu Leu Leu Ala
35 40 45

Arg Asn Cys Lys Ser Leu Ile Ser Leu Lys Met Ser Asp Cys Asp Leu
50 55 60

Ser Asp Leu Ile Gly Phe Leu Gln Thr Ser Lys Ala Leu Gln Glu Ser
65 70 75 80

Ala Gly Arg Arg Phe Phe Arg Ser Arg Arg Val His Gln Val Arg Lys
85 90 95

Gly Xaa Ser His Leu Ala Met Leu Leu Gly Gly Pro Thr Phe Met Gly
100 105 110

Lys Asn Glu Ser Arg Tyr Phe Pro Tyr Pro Arg Arg Leu Lys Thr Gly
115 120 125

Pro Ala Tyr Thr Ser Ser Gln Xaa Lys Xaa Arg His Leu Thr Leu Lys
130 135 140

Pro Asn Leu Arg Val Ser Arg Gly Ala Gly Thr Asn Arg Pro Ile
145 150 155

<210> 25
<211> 486
<212> DNA
<213> Triticum aestivum

<220>
<221> unsure
<222> (197)

<220>
<221> unsure
<222> (275)

<220>
<221> unsure
<222> (289)

<220>
<221> unsure
<222> (298)

<220>
<221> unsure
<222> (334)

<220>
<221> unsure
<222> (346)

<220>
<221> unsure
<222> (399)

<220>
<221> unsure
<222> (441)

<220>
<221> unsure
<222> (463)

<220>
<221> unsure
<222> (466)

<220>
<221> unsure
<222> (470)

<400> 25
cgggaagggg ggaaatcaat ccccatgccc ccacccctcg ccggaccaga tccccggcgg 60
gccggcgcgg agccttaggc ggggatgggc ggggaggccc cggagccgcg gcggctgagc 120
cgcgcgctca gcctggacgg cggcggcgtc ccggaggagg cgctgcacct ggtgctcggc 180
tacgtggacg acccgcncga ccgcgaggcg gcctcgctgg cgtgccgccg ctggcaccac 240
atcgacgcgc tcacgcggaa gcacgtcacc gtgcncttct gctacgccng tgtccccngc 300
gcgcctgctc gcgcgcttcc cgcgcctcga gtcnctcggg gtcaanggca agcccgcgcc 360
gccatgtacg gctcatcccc gacgactggg gcgcctacnc ccgggccctg cgtccctgag 420
ctcgccgccc cgctcgattg nctcaaggcg gctcaacctt gcnccncaan gtcgtcaccg 480
acgaca 486

<210> 26
<211> 134
<212> PRT
<213> Triticum aestivum

<220>
<221> UNSURE
<222> (38)

<220>
<221> UNSURE
<222> (64)

<220>
<221> UNSURE
<222> (69)

<220>
<221> UNSURE
<222> (72)

<220>
<221> UNSURE
<222> (84)

<220>
<221> UNSURE
<222> (88)

<220>
<221> UNSURE
<222> (119)

<220>
<221> UNSURE
<222> (127)..(128)..(129)

<400> 26
Met Gly Gly Glu Ala Pro Glu Pro Arg Arg Leu Ser Arg Ala Leu Ser
1 5 10 15

Leu Asp Gly Gly Gly Val Pro Glu Glu Ala Leu His Leu Val Leu Gly
20 25 30

Tyr Val Asp Asp Pro Xaa Asp Arg Glu Ala Ala Ser Leu Ala Cys Arg
35 40 45

Arg Trp His His Ile Asp Ala Leu Thr Arg Lys His Val Thr Val Xaa
50 55 60

Phe Cys Tyr Ala Xaa Val Pro Xaa Ala Pro Ala Arg Ala Leu Pro Ala
65 70 75 80

Pro Arg Val Xaa Arg Gly Gln Xaa Gln Ala Arg Ala Ala Met Tyr Gly
85 90 95

Ser Ser Pro Thr Thr Gly Ala Pro Thr Pro Gly Pro Cys Val Pro Glu
100 105 110

Leu Ala Ala Pro Leu Asp Xaa Leu Lys Ala Ala Gln Pro Cys Xaa Xaa
115 120 125

Xaa Ser Ser Pro Thr Thr
130

<210> 27
<211> 1074
<212> DNA
<213> Triticum aestivum

<400> 27
gcacgaggta ggattgatgg ctgtagctga aggctgtcct gatttggagt actgggcagt 60
acatgtgtct gacattacaa atgcagctct tgaggctatt ggcgcattca gcaaaaacct 120
gaacgatttc cgacttgtcc tgcttgatag agaggtgcat ataactgaac tgccccttga 180
caacggggtt cgggctttgc tgagaggttg caccaaactc cggaggtttg cattttatgt 240
gagacctgga gctctatcag atattggcct ttcttatgtt ggcgaattta gcaagaccgt 300
ccgctacatg ttgcttggga atgccggggg gtctgatgat ggactgctgg catttgcacg 360
aggatgccca agcttgcaga aattggagct aaggagttgc tgctttagtg aacgtgcatt 420
ggcagttgca gccttacagc tgaagtcact cagatatctt tgggtgcagg gatacaaggc 480
atctcctact ggcaccgatc tcatggcaat ggtacgcccc ttctggaaca ttgagtttat 540
tgcaccaaat caagatgagc cttgcccaga gggtcaggca cagattctgg catactactc 600
tctggctggg gcaaggacag attgtcctca gtcagtaatt cccctccatc cgtcagtggg 660
aagctaaaaa gaccaccacc agtttgactg tacatacatg tttgatgcca gcaaaaccca 720
caatgcggta tagggacatt ccaccttaca gtgccaatta cgggactgaa agctcaagta 780
aaagcgaccc actctgaact gccttggtat cttaggggca acatttttgg gtaagctgtt 840
catctggcca acatggatat ctttgtgtac tacaccattt tgacatggct cggacacgca 900
tttttgtaat aatgtgccca gttgtaatgg catttttctg ttcttgagct ttgcccactg 960
tattgttgtt ctacaaacag tattggatta gttgttgtac catctgtgaa acaatctgca 1020
caatgttatg tttaacccat gaatatcttg aaaaaaaaaa aaaaaaaaaa aaaa 1074

<210> 28
<211> 221
<212> PRT
<213> Triticum aestivum

<400> 28
His Glu Val Gly Leu Met Ala Val Ala Glu Gly Cys Pro Asp Leu Glu
1 5 10 15

Tyr Trp Ala Val His Val Ser Asp Ile Thr Asn Ala Ala Leu Glu Ala
20 25 30

Ile Gly Ala Phe Ser Lys Asn Leu Asn Asp Phe Arg Leu Val Leu Leu
35 40 45

Asp Arg Glu Val His Ile Thr Glu Leu Pro Leu Asp Asn Gly Val Arg
50 55 60

```
Ala Leu Leu Arg Gly Cys Thr Lys Leu Arg Arg Phe Ala Phe Tyr Val
 65                  70                  75                  80

Arg Pro Gly Ala Leu Ser Asp Ile Gly Leu Ser Tyr Val Gly Glu Phe
                 85                  90                  95

Ser Lys Thr Val Arg Tyr Met Leu Leu Gly Asn Ala Gly Gly Ser Asp
            100                 105                 110

Asp Gly Leu Leu Ala Phe Ala Arg Gly Cys Pro Ser Leu Gln Lys Leu
        115                 120                 125

Glu Leu Arg Ser Cys Cys Phe Ser Glu Arg Ala Leu Ala Val Ala Ala
    130                 135                 140

Leu Gln Leu Lys Ser Leu Arg Tyr Leu Trp Val Gln Gly Tyr Lys Ala
145                 150                 155                 160

Ser Pro Thr Gly Thr Asp Leu Met Ala Met Val Arg Pro Phe Trp Asn
                165                 170                 175

Ile Glu Phe Ile Ala Pro Asn Gln Asp Glu Pro Cys Pro Glu Gly Gln
            180                 185                 190

Ala Gln Ile Leu Ala Tyr Tyr Ser Leu Ala Gly Ala Arg Thr Asp Cys
        195                 200                 205

Pro Gln Ser Val Ile Pro Leu His Pro Ser Val Gly Ser
    210                 215                 220
```

<210> 29
<211> 1812
<212> DNA
<213> Oryza sativa

<220>
<221> unsure
<222> (1108)

<400> 29

```
cccccgggct gcaggaattc ggcacgaggt cacgcaacca cggactcctc ctccacctcc   60
gtttcctact ctcttcttca gtttctcacc tctccgcacg agaaaattcg aatccccctt  120
ccggctgctg gttttcgtgc cagaaacagg cgattttacc agtgccagtt agctctcgcc  180
ttcctcctcc tccatcgtgc tactactctg ttcttctgga agaacactgg tctcctcgcc  240
tacctcagtc accactcacc acaccaggtg cgagctataa aaaccggcac gccaaaaatc  300
ttcaaaacca cacagaaacc tcagatctcc gaggcttcca agcgagtcga cgaaaatgcc  360
cgtgatggct ccgaccgcat ctcttctcct ctccccgagg ccgctgccgg cgagccgccg  420
ggtcccctcg ctcccggcgc tctcggcttc cggtcgcctg cgcctccgcc gcgcccgcgc  480
cgacacacgg ctccgcgtgg cggcgccgcc gtccgtcccc ggggaggcgg accaggcgcc  540
cggggagacc gagccgagca cgtcgtcggc cgacgagaag ttcgtgtgga gggaccactg  600
gtaccccgtg tccctcgtcg aggacctcga ccccagcgtg cccaccccgt tccagctcct  660
caaccgcgac ctcgtcatct ggaaggaccc aaaatccggc gagtgggtcg ccctcgacga  720
ccgttgcccc catcgcctcg cgcccctctc ggaggggcgg atcgatgaga cggggtgctt  780
gcagtgctca taccacggct ggtcattcga tggctccggc gcgtgcaccc ggatcccgca  840
ggcggcgccc gaggggccgg aggccaaggc tgtgaggtcg ccgaaggcgt gcgcgatcaa  900
gttccccacc ctcgtctcgc aagggctgct cttcgtgtgg cccgacgaga atgggtggga  960
gaaggccacg gctaccaagc ctccgatgtt accgaaggag tttgaggatc ctgcgttctc 1020
cacggtgacc atccagaggg atctgtacta tggctatgat acattgatgg agaacgtctc 1080
tgatccgtcg catatagaat ttgctcanca caaggtcact ggtcgaagag atcgagccag 1140
```

gcctttgcca ttcaagatgg aatcaagtgg tgcatgggga tattcagggt caaattctgg 1200
aaaccctcgc atcagtgcaa cttttgtggc cccttgctat gcactgaaca aaattgagat 1260
agacacaaag ttacccattt ttggagatca gaaatgggtc atatggattt gctctttcaa 1320
cattccaatg gccccaggga agactcgttc tatagtttgt agtgctcgga acttttttcca 1380
gtttagcatg ccaggaaaag catggtggca gcttgtccct cgatggtatg agcattggac 1440
ttcaaatttg gtctatgatg gtgatatgat agttctgcaa gggcaagaga agattttctt 1500
gtctgcatcg aaggagtctt ctgcagatat taatcagcag tacacaaaga tcacgtttac 1560
acccacgcag gctgaccgtt ttgttttggc attccgggca tggctaagga aatttggtaa 1620
cagccaacct gactggtttg gaaatcctag ccaagaagtg ttgccttcca ctgtcctttc 1680
aaagcgtgag atgctagata gatatgagca gcacacactg aaatgctcat cttgcaaagg 1740
ggcatacaac gccttccaga ctctgcaaaa ggtcttcatg ggagcgacag tggccgttct 1800
attattgctt gc 1812

<210> 30
<211> 485
<212> PRT
<213> Oryza sativa

<220>
<221> UNSURE
<222> (251)

<400> 30

Met Pro Val Met Ala Pro Thr Ala Ser Leu Leu Leu Ser Pro Arg Pro
1 5 10 15

Leu Pro Ala Ser Arg Arg Val Pro Ser Leu Pro Ala Leu Ser Ala Ser
20 25 30

Gly Arg Leu Arg Leu Arg Arg Ala Arg Ala Asp Thr Arg Leu Arg Val
35 40 45

Ala Ala Pro Pro Ser Val Pro Gly Glu Ala Asp Gln Ala Pro Gly Glu
50 55 60

Thr Glu Pro Ser Thr Ser Ser Ala Asp Glu Lys Phe Val Trp Arg Asp
65 70 75 80

His Trp Tyr Pro Val Ser Leu Val Glu Asp Leu Asp Pro Ser Val Pro
85 90 95

Thr Pro Phe Gln Leu Leu Asn Arg Asp Leu Val Ile Trp Lys Asp Pro
100 105 110

Lys Ser Gly Glu Trp Val Ala Leu Asp Asp Arg Cys Pro His Arg Leu
115 120 125

Ala Pro Leu Ser Glu Gly Arg Ile Asp Glu Thr Gly Cys Leu Gln Cys
130 135 140

Ser Tyr His Gly Trp Ser Phe Asp Gly Ser Gly Ala Cys Thr Arg Ile
145 150 155 160

Pro Gln Ala Ala Pro Glu Gly Pro Glu Ala Lys Ala Val Arg Ser Pro
165 170 175

Lys Ala Cys Ala Ile Lys Phe Pro Thr Leu Val Ser Gln Gly Leu Leu
180 185 190

```
Phe Val Trp Pro Asp Glu Asn Gly Trp Glu Lys Ala Thr Ala Thr Lys
        195                 200                 205

Pro Pro Met Leu Pro Lys Glu Phe Glu Asp Pro Ala Phe Ser Thr Val
    210                 215                 220

Thr Ile Gln Arg Asp Leu Tyr Tyr Gly Tyr Asp Thr Leu Met Glu Asn
225                 230                 235                 240

Val Ser Asp Pro Ser His Ile Glu Phe Ala Xaa His Lys Val Thr Gly
                245                 250                 255

Arg Arg Asp Arg Ala Arg Pro Leu Pro Phe Lys Met Glu Ser Ser Gly
            260                 265                 270

Ala Trp Gly Tyr Ser Gly Ser Asn Ser Gly Asn Pro Arg Ile Ser Ala
        275                 280                 285

Thr Phe Val Ala Pro Cys Tyr Ala Leu Asn Lys Ile Glu Ile Asp Thr
    290                 295                 300

Lys Leu Pro Ile Phe Gly Asp Gln Lys Trp Val Ile Trp Ile Cys Ser
305                 310                 315                 320

Phe Asn Ile Pro Met Ala Pro Gly Lys Thr Arg Ser Ile Val Cys Ser
                325                 330                 335

Ala Arg Asn Phe Phe Gln Phe Ser Met Pro Gly Lys Ala Trp Trp Gln
            340                 345                 350

Leu Val Pro Arg Trp Tyr Glu His Trp Thr Ser Asn Leu Val Tyr Asp
        355                 360                 365

Gly Asp Met Ile Val Leu Gln Gly Gln Glu Lys Ile Phe Leu Ser Ala
    370                 375                 380

Ser Lys Glu Ser Ser Ala Asp Ile Asn Gln Gln Tyr Thr Lys Ile Thr
385                 390                 395                 400

Phe Thr Pro Thr Gln Ala Asp Arg Phe Val Leu Ala Phe Arg Ala Trp
                405                 410                 415

Leu Arg Lys Phe Gly Asn Ser Gln Pro Asp Trp Phe Gly Asn Pro Ser
            420                 425                 430

Gln Glu Val Leu Pro Ser Thr Val Leu Ser Lys Arg Glu Met Leu Asp
        435                 440                 445

Arg Tyr Glu Gln His Thr Leu Lys Cys Ser Ser Cys Lys Gly Ala Tyr
    450                 455                 460

Asn Ala Phe Gln Thr Leu Gln Lys Val Phe Met Gly Ala Thr Val Ala
465                 470                 475                 480

Val Leu Leu Leu Leu
                485
```

<210> 31
<211> 1930

<212> DNA
<213> Glycine max

<400> 31
gggaagaaag aaacatttga aacttgcacg actcaactac aatctctctt atgaacacat 60
ctcatttgaa cctctataaa caaattttca aaccttaaca ccttacgaaa atcaactaaa 120
gaaaaccatt gatggcgctc cctcactcca tctctgcctt agccaccaca ctcacactct 180
cctccccaat aaccaaaccc cataaagtta acccctttcc cttttcctcg aaccgaaatt 240
cacaattttt aacgaaacaa acgcgaccca gaagcagaag aaacctctcc ctaacccctg 300
cacgcgttgc ggcgccaccc tcaacggttg aagccgatcg attataccca gaggccgaaa 360
ataacgaaac tgaggaagag tttagcgacg agagctcttc ctctaaattc acttggaggg 420
atcactggta ccctgtctcg ttaattgaag atctgaaccc tctcttgccc acaccgtttc 480
agcttctggg tcgtgaaatc gtgctctggt acgacaagtc catttcccaa tgggttgctt 540
ttgatgacaa atgcccccat cgtcttgccc ctttatctga agggaggata gatgaagatg 600
ggaagttgca gtgttcttat catgggtggt cttttgatgg gtgtggatct tgtgttaaga 660
ttcctcaggc ttcatctgaa ggccccgaag cacgtgctat tggatctcct aaagcatgtg 720
ccactaggtt ccctaccttg gtgtcccagg gtttgctctt tgtatgggct gatgagaatg 780
gttgggagaa agcaaaggcc tccaaccctc caatgtttcc tgatgacttt gacaaaccgg 840
agtttcccac ggtcaacata cagcgtgatt tgttctatgg ttacgatact cttatggaga 900
atgtctctga tccttctcac attgagtttg ctcatcacaa ggtcacggga aggagagaca 960
gagccaaacc tctgccattc aagatggatt ctcgtggttc atggggcttc tctggagcta 1020
atgaagggaa cccacagatc agtgccaagt ttgttgcacc atgttatatg atgaacaaga 1080
ttgagattga taccaaactc cctgtagttg gtgaccagaa atgggtagta tggatatgtt 1140
ccttcaatgt ccccatggca cctggtaaga ctcgctccat tgtttgcagt gctcgaaact 1200
tcttccagtt ctcagtgcca gggcctgcct ggtggcaagt caactgagta atcttactgt 1260
ttgcattcaa ttttaaacaa tgcatacatg taactcaggt cgttcctaga tggtatgagc 1320
attggacttc aaataaggta tatgatggag acatgattgt ccttcaaggt caagagaaaa 1380
tcttcctttc agaaaccaag gaaggtggtg acattaacaa acagtacaca aacatcacct 1440
tcacaccaac acaggcagat cgctttgtct tggcattccg aaattggctg aggcgacatg 1500
gcaatggcca accagaatgg tttggaaaca gcagcgacca gccattgcca tcaactgtgt 1560
tatcaaaacg tcagatgttg gatagatttg aacagcacac tctcaagtgt tcatcatgta 1620
aagcagcata tgagggattc caaacatggc agaaagtcct aattggggca acagttgtgt 1680
tttgtgcaac atcagggatc ccatcagatt tccagttgcg tgtacttttg gctggactcg 1740
cagttgtcag cgcagccata gcttttgccc taaaccaact ccaaaagaat tttgaattcg 1800
tggattacgt gcatgcggaa atcgattaag cacgtccctc caaaggaact tcaactagtt 1860
agttgtaaat agagttgaag acaagtacat gtacactagt attttgatga aaagagctca 1920
aatctacctt 1930

<210> 32
<211> 563
<212> PRT
<213> Glycine max

<400> 32
Met Ala Leu Pro His Ser Ile Ser Ala Leu Ala Thr Thr Leu Thr Leu
1 5 10 15

Ser Ser Pro Ile Thr Lys Pro His Lys Val Asn Pro Phe Pro Phe Ser
20 25 30

Ser Asn Arg Asn Ser Gln Phe Leu Thr Lys Gln Thr Arg Pro Arg Ser
35 40 45

Arg Arg Asn Leu Ser Leu Thr Pro Ala Arg Val Ala Ala Pro Pro Ser
50 55 60

Thr Val Glu Ala Asp Arg Leu Tyr Pro Glu Ala Glu Asn Asn Glu Thr
65 70 75 80

```
Glu Glu Glu Phe Ser Asp Glu Ser Ser Ser Lys Phe Thr Trp Arg Asp
                  85                  90                  95

His Trp Tyr Pro Val Ser Leu Ile Glu Asp Leu Asn Pro Leu Leu Pro
             100                 105                 110

Thr Pro Phe Gln Leu Leu Gly Arg Glu Ile Val Leu Trp Tyr Asp Lys
         115                 120                 125

Ser Ile Ser Gln Trp Val Ala Phe Asp Asp Lys Cys Pro His Arg Leu
     130                 135                 140

Ala Pro Leu Ser Glu Gly Arg Ile Asp Glu Asp Gly Lys Leu Gln Cys
145                 150                 155                 160

Ser Tyr His Gly Trp Ser Phe Asp Gly Cys Gly Ser Cys Val Lys Ile
                 165                 170                 175

Pro Gln Ala Ser Ser Glu Gly Pro Glu Ala Arg Ala Ile Gly Ser Pro
             180                 185                 190

Lys Ala Cys Ala Thr Arg Phe Pro Thr Leu Val Ser Gln Gly Leu Leu
         195                 200                 205

Phe Val Trp Ala Asp Glu Asn Gly Trp Glu Lys Ala Lys Ala Ser Asn
     210                 215                 220

Pro Pro Met Phe Pro Asp Asp Phe Asp Lys Pro Glu Phe Pro Thr Val
225                 230                 235                 240

Asn Ile Gln Arg Asp Leu Phe Tyr Gly Tyr Asp Thr Leu Met Glu Asn
                 245                 250                 255

Val Ser Asp Pro Ser His Ile Glu Phe Ala His His Lys Val Thr Gly
             260                 265                 270

Arg Arg Asp Arg Ala Lys Pro Leu Pro Phe Lys Met Asp Ser Arg Gly
         275                 280                 285

Ser Trp Gly Phe Ser Gly Ala Asn Glu Gly Asn Pro Gln Ile Ser Ala
     290                 295                 300

Lys Phe Val Ala Pro Cys Tyr Met Met Asn Lys Ile Glu Ile Asp Thr
305                 310                 315                 320

Lys Leu Pro Val Val Gly Asp Gln Lys Trp Val Val Trp Ile Cys Ser
                 325                 330                 335

Phe Asn Val Pro Met Ala Pro Gly Lys Thr Arg Ser Ile Val Cys Ser
             340                 345                 350

Ala Arg Asn Phe Phe Gln Phe Ser Val Pro Gly Pro Ala Trp Trp Gln
         355                 360                 365

Val Asn Val Ile Leu Leu Phe Ala Phe Asn Phe Lys Gln Cys Ile His
     370                 375                 380

Val Thr Gln Val Val Pro Arg Trp Tyr Glu His Trp Thr Ser Asn Lys
385                 390                 395                 400
```

```
Val Tyr Asp Gly Asp Met Ile Val Leu Gln Gly Gln Glu Lys Ile Phe
                405                 410                 415

Leu Ser Glu Thr Lys Glu Gly Gly Asp Ile Asn Lys Gln Tyr Thr Asn
            420                 425                 430

Ile Thr Phe Thr Pro Thr Gln Ala Asp Arg Phe Val Leu Ala Phe Arg
        435                 440                 445

Asn Trp Leu Arg Arg His Gly Asn Gly Gln Pro Glu Trp Phe Gly Asn
    450                 455                 460

Ser Ser Asp Gln Pro Leu Pro Ser Thr Val Leu Ser Lys Arg Gln Met
465                 470                 475                 480

Leu Asp Arg Phe Glu Gln His Thr Leu Lys Cys Ser Ser Cys Lys Ala
                485                 490                 495

Ala Tyr Glu Gly Phe Gln Thr Trp Gln Lys Val Leu Ile Gly Ala Thr
            500                 505                 510

Val Val Phe Cys Ala Thr Ser Gly Ile Pro Ser Asp Phe Gln Leu Arg
        515                 520                 525

Val Leu Leu Ala Gly Leu Ala Val Val Ser Ala Ala Ile Ala Phe Ala
    530                 535                 540

Leu Asn Gln Leu Gln Lys Asn Phe Glu Phe Val Asp Tyr Val His Ala
545                 550                 555                 560

Glu Ile Asp
```

<210> 33
<211> 555
<212> DNA
<213> Triticum aestivum

<220>
<221> unsure
<222> (228)

<220>
<221> unsure
<222> (252)

<220>
<221> unsure
<222> (354)

<220>
<221> unsure
<222> (369)

<220>
<221> unsure
<222> (402)

<220>
<221> unsure
<222> (412)

<220>
<221> unsure
<222> (415)

<220>
<221> unsure
<222> (441)

<220>
<221> unsure
<222> (460)

<220>
<221> unsure
<222> (467)

<220>
<221> unsure
<222> (482)

<220>
<221> unsure
<222> (504)

<220>
<221> unsure
<222> (506)

<220>
<221> unsure
<222> (519)..(520)

<220>
<221> unsure
<222> (524)

<220>
<221> unsure
<222> (539)

<220>
<221> unsure
<222> (541)

<220>
<221> unsure
<222> (544)

<220>
<221> unsure
<222> (555)

<400> 33
tccacccacg ccgtcgatca cccggtggtc accaccggcg acatggatcc cctccgccta 60
ctcctccccc gcgcccaggc ccagcccttg cttccgctcc ccaccggcgt ccaagcaccg 120
agcgtcaggc cccaactcgt cccgcggcga cgggcgcgcc gccaccgcaa cggggccgcg 180

cggatgctgc cggcctcggc cgtggcgtcc gagtcgccgt ggacgganca ggagccgcca 240
tccggggaga angaggagcg gttcgactgg ctggaccagt ggtacccctt cgcccccgtg 300
gaggacctgg acccggcgcg cccacggcaa atggtgctgg gatccgcgtg gtanctggta 360
caacgcggng ccggcgaatg gcgctgttca caccgtgccc gnacgcctgg cncgnctcga 420
gggcgcatca caaaaggcgg ncagtcgtta cacgggtggn ctcacgncgc gggctgaatt 480
ancccaggcc cgcctcggca acgngnaaca aaacagggnn gtgnttaacc gtctgtgana 540
naanttgtgt ctccn 555

<210> 34
<211> 144
<212> PRT
<213> Triticum aestivum

<220>
<221> UNSURE
<222> (62)

<220>
<221> UNSURE
<222> (70)

<220>
<221> UNSURE
<222> (104)

<220>
<221> UNSURE
<222> (124)..(125)

<220>
<221> UNSURE
<222> (140)

<220>
<221> UNSURE
<222> (142)

<400> 34
Met Asp Pro Leu Arg Leu Leu Leu Pro Arg Ala Gln Ala Gln Pro Leu
1 5 10 15

Leu Pro Leu Pro Thr Gly Val Gln Ala Pro Ser Val Arg Pro Gln Leu
20 25 30

Val Pro Arg Arg Arg Ala Arg Arg His Arg Asn Gly Ala Ala Arg Met
35 40 45

Leu Pro Ala Ser Ala Val Ala Ser Glu Ser Pro Trp Thr Xaa Gln Glu
50 55 60

Pro Pro Ser Gly Glu Xaa Glu Glu Arg Phe Asp Trp Leu Asp Gln Trp
65 70 75 80

Tyr Pro Phe Ala Pro Val Glu Asp Leu Asp Pro Ala Arg Pro Arg Gln
85 90 95

Met Val Leu Gly Ser Ala Trp Xaa Leu Val Gln Arg Gly Ala Gly Glu
100 105 110

Trp Arg Cys Ser His Arg Ala Arg Thr Pro Gly Xaa Xaa Arg Gly Arg
115 120 125

Ile Thr Lys Gly Gly Gln Ser Leu His Gly Trp Xaa His Xaa Ala Gly
130 135 140

<210> 35
<211> 1864
<212> DNA
<213> Triticum aestivum

<400> 35
gcacgagggc aatgttctag aagcaccgaa gcaccgagag ataagtggca ctagtacaaa 60
gctggagcga ggaagatctc ggccccaaca aaacctcgga ccccttccct ccacacgatc 120
ccgaggaagg aaggaaaggc agacgaaatg ccggtgctgg cgatgccgtc cgcctccctc 180
cccctcctct cccccggggc accggccgct gctgcgcccg tcgaccctcc cggcctcccg 240
tctcggcagc ggcatcctcc gcgtggccgc gccgacgtcg gtccccggcg aggcggagcg 300
ggcggaggag ccgagcacga gcacgagcac ctcgcctgaa tcgtccgggg agaagttcgt 360
gtggcgggac cactggtacc cggtctcgct cgtggaggac ctggacccgc gcgtgcccac 420
cccgttccag ctcctcaacc gcgacctcgt catctggaac gaccccaact ccggcgactg 480
ggtcgcgctc gacgaccgct gcccgcaccg cctcgccccg ctctcggagg ggcggatcga 540
cgagacgggc ggcctgcagt gctcctacca cggctggtcc ttcgacggct ccggcgcctg 600
caccaggatc ccgcaggccg cgcccgaggg gcccgaggcc cgggcggtgc gctcgcccag 660
ggcctgcgcc accaagttcc ccaccctcct ctcccagggc ctgctcttcg tctggcctga 720
cgagaatgga tgggacaagg ccaaggccac caagcctcca atgctgccga aggagttcga 780
tgacccggcc ttctccaccg tgacgatcca gagggacctc ttctatgggt atgacacgtt 840
gatggagaac gtctctgatc cctcgcatat agaatttgct caccacaagg tcactggacg 900
aagagataga gccaagcctt tgccatttaa aatggaatca agtggcgcat ggggatattc 960
aggggcaaat accggcaatc ctcgcatcac tgcaactttc gaggcccctt gctatgcact 1020
gaacaaaata gagattgaca ccaaattacc gattgtggga gatcagaaat gggtcatatg 1080
gatttgctcc ttcaacattc caatggcccc agggaaaact cgttctattg tctgtagtgc 1140
tcgaaacttt ttccagttta caatgccagg aaaggcatgg tggcagtttg tccctcgatg 1200
gtacgaacat tggacctcaa atttggtcta cgacggcgat atgatcgtgc ttcaaggcca 1260
agagaaggtt ttcctgtctg catccaagga gtcgtctgca gatgttaatc agcagtacac 1320
aaagctcaca ttcacaccca cacaggccga ccgatttgtt ttagcattcc gggcatggct 1380
acgaaaattc ggaaatagcc agcctgactg gtatggaagt cctagccaag atgcattgcc 1440
ttctacggtc ctttcaaagc gagagatgct agacagatac gagcagcaca cgctgaaatg 1500
ctcgtcctgc agaggagcgc acaaggcctt tcagactttg cagaaggtgt tcatgggggc 1560
gacggtggtg tttggcgcga catccgggat ccctgcggat gttcagctca gaatattgct 1620
cggtgccggt gctctggtca gcgccgctct ggcctatgtc ttctacgacc gccagaagca 1680
tttcgtgttt gtggactacg tgcacgctga cattgattga ttagggagat aaacattagt 1740
tatttttgtg aggatctggt gtggtgtggt gtggagacat cccacgatca atcatgtgca 1800
taacctagcc aaggagtaca tatagctttc agtgggtaca tgagattggc ccagtatgtt 1860
gttt 1864

<210> 36
<211> 487
<212> PRT
<213> Triticum aestivum

<400> 36
Leu Arg Val Ala Ala Pro Thr Ser Val Pro Gly Glu Ala Glu Arg Ala
1 5 10 15

Glu Glu Pro Ser Thr Ser Thr Ser Thr Ser Pro Glu Ser Ser Gly Glu
20 25 30

Lys Phe Val Trp Arg Asp His Trp Tyr Pro Val Ser Leu Val Glu Asp
35 40 45

```
Leu Asp Pro Arg Val Pro Thr Pro Phe Gln Leu Leu Asn Arg Asp Leu
     50                  55                  60

Val Ile Trp Asn Asp Pro Asn Ser Gly Asp Trp Val Ala Leu Asp Asp
 65                  70                  75                  80

Arg Cys Pro His Arg Leu Ala Pro Leu Ser Glu Gly Arg Ile Asp Glu
                 85                  90                  95

Thr Gly Gly Leu Gln Cys Ser Tyr His Gly Trp Ser Phe Asp Gly Ser
            100                 105                 110

Gly Ala Cys Thr Arg Ile Pro Gln Ala Ala Pro Glu Gly Pro Glu Ala
        115                 120                 125

Arg Ala Val Arg Ser Pro Arg Ala Cys Ala Thr Lys Phe Pro Thr Leu
    130                 135                 140

Leu Ser Gln Gly Leu Leu Phe Val Trp Pro Asp Glu Asn Gly Trp Asp
145                 150                 155                 160

Lys Ala Lys Ala Thr Lys Pro Pro Met Leu Pro Lys Glu Phe Asp Asp
                165                 170                 175

Pro Ala Phe Ser Thr Val Thr Ile Gln Arg Asp Leu Phe Tyr Gly Tyr
            180                 185                 190

Asp Thr Leu Met Glu Asn Val Ser Asp Pro Ser His Ile Glu Phe Ala
        195                 200                 205

His His Lys Val Thr Gly Arg Arg Asp Arg Ala Lys Pro Leu Pro Phe
    210                 215                 220

Lys Met Glu Ser Ser Gly Ala Trp Gly Tyr Ser Gly Ala Asn Thr Gly
225                 230                 235                 240

Asn Pro Arg Ile Thr Ala Thr Phe Glu Ala Pro Cys Tyr Ala Leu Asn
                245                 250                 255

Lys Ile Glu Ile Asp Thr Lys Leu Pro Ile Val Gly Asp Gln Lys Trp
            260                 265                 270

Val Ile Trp Ile Cys Ser Phe Asn Ile Pro Met Ala Pro Gly Lys Thr
        275                 280                 285

Arg Ser Ile Val Cys Ser Ala Arg Asn Phe Phe Gln Phe Thr Met Pro
    290                 295                 300

Gly Lys Ala Trp Trp Gln Phe Val Pro Arg Trp Tyr Glu His Trp Thr
305                 310                 315                 320

Ser Asn Leu Val Tyr Asp Gly Asp Met Ile Val Leu Gln Gly Gln Glu
                325                 330                 335

Lys Val Phe Leu Ser Ala Ser Lys Glu Ser Ser Ala Asp Val Asn Gln
            340                 345                 350

Gln Tyr Thr Lys Leu Thr Phe Thr Pro Thr Gln Ala Asp Arg Phe Val
        355                 360                 365
```

```
Leu Ala Phe Arg Ala Trp Leu Arg Lys Phe Gly Asn Ser Gln Pro Asp
    370                 375                 380

Trp Tyr Gly Ser Pro Ser Gln Asp Ala Leu Pro Ser Thr Val Leu Ser
385                 390                 395                 400

Lys Arg Glu Met Leu Asp Arg Tyr Glu Gln His Thr Leu Lys Cys Ser
                405                 410                 415

Ser Cys Arg Gly Ala His Lys Ala Phe Gln Thr Leu Gln Lys Val Phe
            420                 425                 430

Met Gly Ala Thr Val Val Phe Gly Ala Thr Ser Gly Ile Pro Ala Asp
        435                 440                 445

Val Gln Leu Arg Ile Leu Leu Gly Ala Gly Ala Leu Val Ser Ala Ala
    450                 455                 460

Leu Ala Tyr Val Phe Tyr Asp Arg Gln Lys His Phe Val Phe Val Asp
465                 470                 475                 480

Tyr Val His Ala Asp Ile Asp
                485
```

<210> 37
<211> 592
<212> PRT
<213> Arabidopsis thaliana

<400> 37

```
Met Glu Asp Pro Asp Ile Lys Arg Cys Lys Leu Ser Cys Val Ala Thr
  1               5                  10                  15

Val Asp Asp Val Ile Glu Gln Val Met Thr Tyr Ile Thr Asp Pro Lys
             20                  25                  30

Asp Arg Asp Ser Ala Ser Leu Val Cys Arg Arg Trp Phe Lys Ile Asp
         35                  40                  45

Ser Glu Thr Arg Glu His Val Thr Met Ala Leu Cys Tyr Thr Ala Thr
     50                  55                  60

Pro Asp Arg Leu Ser Arg Arg Phe Pro Asn Leu Arg Ser Leu Lys Leu
 65                  70                  75                  80

Lys Gly Lys Pro Arg Ala Ala Met Phe Asn Leu Ile Pro Glu Asn Trp
                85                  90                  95

Gly Gly Tyr Val Thr Pro Trp Val Thr Glu Ile Ser Asn Asn Leu Arg
            100                 105                 110

Gln Leu Lys Ser Val His Phe Arg Arg Met Ile Val Ser Asp Leu Asp
        115                 120                 125

Leu Asp Arg Leu Ala Lys Ala Arg Ala Asp Asp Leu Glu Thr Leu Lys
    130                 135                 140

Leu Asp Lys Cys Ser Gly Phe Thr Thr Asp Gly Leu Leu Ser Ile Val
145                 150                 155                 160
```

```
Thr His Cys Arg Lys Ile Lys Thr Leu Leu Met Glu Glu Ser Ser Phe
                165                 170                 175

Ser Glu Lys Asp Gly Lys Trp Leu His Glu Leu Ala Gln His Asn Thr
            180                 185                 190

Ser Leu Glu Val Leu Asn Phe Tyr Met Thr Glu Phe Ala Lys Ile Ser
        195                 200                 205

Pro Lys Asp Leu Glu Thr Ile Ala Arg Asn Cys Arg Ser Leu Val Ser
    210                 215                 220

Val Lys Val Gly Asp Phe Glu Ile Leu Glu Leu Val Gly Phe Phe Lys
225                 230                 235                 240

Ala Ala Ala Asn Leu Glu Glu Phe Cys Gly Gly Ser Leu Asn Glu Asp
                245                 250                 255

Ile Gly Met Pro Glu Lys Tyr Met Asn Leu Val Phe Pro Arg Lys Leu
            260                 265                 270

Cys Arg Leu Gly Leu Ser Tyr Met Gly Pro Asn Glu Met Pro Ile Leu
        275                 280                 285

Phe Pro Phe Ala Ala Gln Ile Arg Lys Leu Asp Leu Leu Tyr Ala Leu
    290                 295                 300

Leu Glu Thr Glu Asp His Cys Thr Leu Ile Gln Lys Cys Pro Asn Leu
305                 310                 315                 320

Glu Val Leu Glu Thr Arg Asn Val Ile Gly Asp Arg Gly Leu Glu Val
                325                 330                 335

Leu Ala Gln Tyr Cys Lys Gln Leu Lys Arg Leu Arg Ile Glu Arg Gly
            340                 345                 350

Ala Asp Glu Gln Gly Met Glu Asp Glu Glu Gly Leu Val Ser Gln Arg
        355                 360                 365

Gly Leu Ile Ala Leu Ala Gln Gly Cys Gln Glu Leu Glu Tyr Met Ala
    370                 375                 380

Val Tyr Val Ser Asp Ile Thr Asn Glu Ser Leu Glu Ser Ile Gly Thr
385                 390                 395                 400

Tyr Leu Lys Asn Leu Cys Asp Phe Arg Leu Val Leu Leu Asp Arg Glu
                405                 410                 415

Glu Arg Ile Thr Asp Leu Pro Leu Asp Asn Gly Val Arg Ser Leu Leu
            420                 425                 430

Ile Gly Cys Lys Lys Leu Arg Arg Phe Ala Phe Tyr Leu Arg Gln Gly
        435                 440                 445

Gly Leu Thr Asp Leu Gly Leu Ser Tyr Ile Gly Gln Tyr Ser Pro Asn
    450                 455                 460

Val Arg Trp Met Leu Leu Gly Tyr Val Gly Glu Ser Asp Glu Gly Leu
465                 470                 475                 480
```

Met Glu Phe Ser Arg Gly Cys Pro Asn Leu Gln Lys Leu Glu Met Arg
485 490 495

Gly Cys Cys Phe Ser Glu Arg Ala Ile Ala Ala Ala Val Thr Lys Leu
500 505 510

Pro Ser Leu Arg Tyr Leu Trp Val Gln Gly Tyr Arg Ala Ser Met Thr
515 520 525

Gly Gln Asp Leu Met Gln Met Ala Arg Pro Tyr Trp Asn Ile Glu Leu
530 535 540

Ile Pro Ser Arg Arg Val Pro Glu Val Asn Gln Gln Gly Glu Ile Arg
545 550 555 560

Glu Met Glu His Pro Ala His Ile Leu Ala Tyr Tyr Ser Leu Ala Gly
565 570 575

Gln Arg Thr Asp Cys Pro Thr Thr Val Arg Val Leu Lys Glu Pro Ile
580 585 590

<210> 38
<211> 520
<212> PRT
<213> Zea mays

<400> 38
Met Arg Ala Thr Ile Pro Ala Leu Ser Leu Leu Val Thr Pro Arg Leu
1 5 10 15

Pro Ser Leu Ala Val Pro Leu Ala Gly Gly Arg Leu Arg Glu Gly Gly
20 25 30

Arg Ser Arg Thr Arg Leu Arg Val Ala Ala Pro Thr Ser Val Pro Gly
35 40 45

Glu Ala Ala Glu Gln Ala Glu Pro Ser Thr Ser Ala Pro Glu Ser Gly
50 55 60

Glu Lys Phe Ser Trp Arg Asp His Trp Tyr Pro Val Ser Leu Val Glu
65 70 75 80

Asp Leu Asp Pro Ser Arg Pro Thr Pro Phe Gln Leu Leu Asn Arg Asp
85 90 95

Leu Val Ile Trp Lys Glu Pro Lys Ser Gly Glu Trp Val Ala Leu Asp
100 105 110

Asp Arg Cys Pro His Arg Leu Ala Pro Leu Ser Glu Gly Arg Ile Asp
115 120 125

Glu Thr Gly Cys Leu Gln Cys Ser Tyr His Gly Trp Ser Phe Asp Gly
130 135 140

Ser Gly Ala Cys Thr Lys Ile Pro Gln Ala Met Pro Glu Gly Pro Glu
145 150 155 160

Ala Arg Ala Val Arg Ser Pro Lys Ala Cys Ala Ile Lys Phe Pro Thr
165 170 175

Leu Val Ser Gln Gly Leu Leu Phe Val Trp Pro Asp Glu Asn Gly Trp
180 185 190

Glu Lys Ala Ala Ala Thr Lys Pro Pro Met Leu Pro Lys Glu Phe Glu
195 200 205

Asp Pro Ala Phe Ser Thr Val Thr Ile Gln Arg Asp Leu Phe Tyr Gly
210 215 220

Tyr Asp Thr Leu Met Glu Asn Val Ser Asp Pro Ser His Ile Glu Phe
225 230 235 240

Ala His His Lys Val Thr Gly Arg Arg Asp Arg Ala Arg Pro Leu Thr
245 250 255

Phe Arg Met Glu Ser Ser Gly Ala Trp Gly Tyr Ser Gly Ala Asn Ser
260 265 270

Gly Asn Pro Arg Ile Thr Ala Thr Phe Glu Ala Pro Cys Tyr Ala Leu
275 280 285

Asn Lys Ile Glu Ile Asp Thr Lys Leu Pro Ile Phe Gly Asp Gln Lys
290 295 300

Trp Val Ile Trp Ile Cys Ser Phe Asn Ile Pro Met Ala Pro Gly Lys
305 310 315 320

Thr Arg Ser Ile Val Cys Ser Ala Arg Asn Phe Phe Gln Phe Thr Met
325 330 335

Pro Gly Lys Ala Trp Trp Gln Leu Val Pro Arg Trp Tyr Glu His Trp
340 345 350

Thr Ser Asn Leu Val Tyr Asp Gly Asp Met Ile Val Leu Gln Gly Gln
355 360 365

Glu Lys Ile Phe Leu Ala Ala Thr Lys Glu Ser Ser Thr Asp Ile Asn
370 375 380

Gln Gln Tyr Thr Lys Ile Thr Phe Thr Pro Thr Gln Ala Asp Arg Phe
385 390 395 400

Val Leu Ala Cys Arg Thr Trp Leu Arg Lys Phe Gly Asn Ser Gln Pro
405 410 415

Glu Trp Phe Gly Asn Pro Thr Gln Glu Ala Leu Pro Ser Thr Val Leu
420 425 430

Ser Lys Arg Glu Met Leu Asp Arg Tyr Glu Gln Leu Ser Leu Lys Cys
435 440 445

Ser Ser Cys Lys Gly Ala Tyr Asn Ala Phe Gln Asn Leu Gln Lys Val
450 455 460

Phe Met Gly Ala Thr Val Val Cys Cys Ala Ala Ala Gly Ile Pro Pro
465 470 475 480

Asp Val Gln Leu Arg Leu Leu Ile Gly Ala Ala Ala Leu Val Ser Ala
485 490 495

Ala Ile Ala Tyr Ala Phe His Glu Leu Gln Lys Asn Phe Val Phe Val
500 505 510

Asp Tyr Val His Ala Asp Ile Asp
515 520